The demand must be filed directly with the competent International Preliminary Examining Authority, unless otherwise provided for in an agreement with the competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/EPO

PCT

DEMAND

CHAPTER II

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only	
Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or Agent's file reference HKA-009.25
International Application No. PCT/US97/06509	International filing date *(day/month/year)* 17 April 1997 (17.04.97)	(Earliest) Priority Date *(day/month/year)* 17 April 1996 (17.04.96)
Title of invention A Combinatorial Protecting Group Strategy for Multifunctional Molecules		

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*	Telephone No.: 617-345-9320
KÖSTER, Hubert 1640 Monument Street Concord, Massachusetts 02110 United States of America	Facsimile No.: 617-345-9377
	Teleprinter No.:

State *(i.e. country)* of nationality: GR	State *(i.e. country)* of residence: United States of America

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*	
State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*	
State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:

[ ] Further applicants are indicated on a continuation sheet.

Internati nal application No.
PCT/US97/06509

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is h reby appointed and any earlier appointment of (an) agent(s)/common representativ is h reby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and Address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ARNOLD, Beth E.
Patent Group
F ley, Hoag & Eliot LLP
One Post Office Square
Boston, Massachusetts 02110
United States of America

Telephone No.:
(617) 832-1000

Facsimile No.:
(617) 832-7000

Teleprinter No.:

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV STATEMENT CONCERNING AMENDMENTS

The applicant wishes the International Preliminary Examining Authority*

(i) [X] to start the international preliminary examination on the basis of the international application as originally filed.

(ii) [ ] to take into account the amendments under Article 34 of

[ ] the description (amendments attached).

[ ] the claims (amendments attached).

[ ] the drawings (amendments attached).

(iii) [ ] to take into account any amendments of the claims under Article 19 filed with the International Bureau (a c py is attached).

(iv) [ ] to disregard any amendments of the claims made under Article 19 and to consider them as reversed.

(v) [ ] to postpone the start of the international preliminary examination until the expiration of 20 months from the priority date unless that Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Box No. V ELECTION OF STATES

[X] The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)* except .....

.....

.....

.....

(If the applicant does not wish to elect certain eligible States, the name(s) or country code(s) of those States must be indicated above.)

International application No.
PCT/US97/06509

Box No. VI CHECK LIST

The demand is accompanied by the following documents for the purposes of international preliminary examination:		For International Preliminary Examining Authority use only: received	not received
1. amendments under Article 34			
description	: sheets	☐	☐
claims	: sheets	☐	☐
drawings	: sheets	☐	☐
2. letter accompanying amendments under Article 34	: sheets	☐	☐
3. copy of amendments under Article 19	: sheets	☐	☐
4. copy of statement under Article 19	: sheets	☐	☐
5. other *(specify):*	: sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [ ] separate signed power of attorney
2. [ ] one copy of each executed power of attorney
3. [ ] statement explaining lack of signature
4. [X] fee calculation sheet
5. [X] other (specify): Transmittal Letter, EPA/EPO/OEB Form 1037, Authorization to Charge Deposit Account No. 283 002 07, and a copy of the Transmittal Letter.

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Beth E. Arnold

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5 below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PCT

CHAPTER II

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No. PCT/US97/06509

For International Preliminary Examining Authority use only

Date stamp of the IPEA

Applicant's or agent's file reference HKA-009.25

Applicant

KÖSTER, Hubert

Calculation of prescribed fees

1.	Preliminary examination fee.....	DEM 3,000	P
2.	Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)*.....	DEM 292	H
3.	Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box.....	DEM 3,292	TOTAL

Mode of Payment

[X]	authorization to charge deposit account with the IPEA (see below)	[ ]	cash
[ ]	cheque	[ ]	revenue stamps
[ ]	postal money order	[ ]	coupons
[ ]	bank draft	[ ]	others *(specify)*:

Deposit Account Authorization *(this mode of payment may not be available at all IPEAs)*

The IPEA/ EPO [X] is hereby authorized to charge the total fees indicated above to my deposit account.

[X] *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

283 002 07	17 November 1997	
Deposit Account Number	Date *(day/month/year)*	Signature

See Notes to the fee calculation sheet

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference: HKA-009.25 *(if desired) (12 characters maximum)*

Box No. I TITLE OF INVENTION
A Combinatorial Protecting Group Strategy For Multifunctional Molecules

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address in this Box is the applicant's State (i.e., country) of residence if no State of residence is indicated below.)*

Köster Hubert
1640 Monument Street
Concord, Massachusetts 02110
United States of America

This person is also inventor. [X]

Telephone No.
617-345-9320

Facsimile No.
617-345-9377

Teleprinter No.

State *(i.e. country)* of nationality:
GR

State *(i.e. country)* of residence:
US

The person is applicant for the purposes of: [X] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

B x No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address in this Box is the applicant's State (i.e., country) of residence if no State of residence is indicated below.)*

Leikauf Eckart
Rudemannweg 11
21107 Hamburg
GERMANY

This person is:

[ ] applicant only

[ ] applicant and inventor

[X] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

The person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on a continuation sheet.

B x No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: [X] agent [ ] common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ARNOLD, Beth E., BLODGETT-FORD, Sayoko J., CELLA, Charles H., CLAUSS, Isabelle, GORECKI, John C., KELLY, Edward J., MUIRHEAD, Donald W., VINCENT, Matthew P.
Foley, Hoag & Eliot LLP
One Post Office Square
Boston, Massachusetts 02109 UNITED STATES OF AMERICA

Telephone No.
(617) 832-1000

Facsimile No.
(617) 832-7000

Teleprinter No.

[ ]Mark this check-box when no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent

Box No. V DESIGNATION OF STATES

The following designations are hereby m[illegible]nder Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

[X] **AP** **ARIPO Patent:** KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT

[X] **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Krgyzstan, KZ Kazakstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

[X] **EP** **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

[X] **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* . . . . . .

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

[X] **AL** Albania . . . . . .
[X] **AM** Armenia . . . . . .
[X] **AT** Austria . . . . . .
[X] **AU** Australia . . . . . .
[X] **AZ** Azerbaijan
[X] **BA** Bosnia and Herzegovina
[X] **BB** Barbados
[X] **BG** Bulgaria . . . . . .
[X] **BR** Brazil . . . . . .
[X] **BY** Belarus
[X] **CA** Canada
[X] **CH** and **LI** Switzerland and Liechtenstein
[X] **CN** China . . . . . .
[X] **CU** Cuba . . . . . .
[X] **CZ** Czech Republic . . . . . .
[X] **DE** Germany . . . . . .
[X] **DK** Denmark . . . . . .
[X] **EE** Estonia . . . . . .
[X] **ES** Spain . . . . . .
[X] **FI** Finland . . . . . .
[X] **GB** United Kingdom
[X] **GE** Georgia . . . . . .
[X] **HU** Hungary . . . . . .
[X] **IL** Israel
[X] **IS** Iceland
[X] **JP** Japan . . . . . .
[X] **KE** Kenya . . . . . .
[X] **KG** Kyrgyzstan . . . . . .
[X] **KP** Democratic People's Republic of Korea . . . . . .
[X] **KR** Republic of Korea . . . . . .
[X] **KZ** Kazakstan . . . . . .
[X] **LC** Saint Lucia
[X] **LK** Sri Lanka
[X] **LR** Liberia
[X] **LS** Lesotho . . . . . .
[X] **LT** Lithuania
[X] **LU** Luxembourg . . . . . .
[X] **LV** Latvia
[X] **MD** Republic of Moldova . . . . . .
[X] **MG** Madagascar . . . . . .
[X] **MK** The former Yugoslav Republic of Macedonia . . . . . .
[X] **MN** Mongolia
[X] **MW** Malawi . . . . . .
[X] **MX** Mexico . . . . . .
[X] **NO** Norway
[X] **NZ** New Zealand . . . . . .
[X] **PL** Poland . . . . . .
[X] **PT** Portugal . . . . . .
[X] **RO** Romania
[X] **RU** Russian Federation . . . . . .
[X] **SD** Sudan
[X] **SE** Sweden
[X] **SG** Singapore
[X] **SI** Slovenia . . . . . .
[X] **SK** Slovakia . . . . . .
[X] **TJ** Tajikistan . . . . . .
[X] **TM** Turkmenistan . . . . . .
[X] **TR** Turkey . . . . . .
[X] **TT** Trinidad and Tobago . . . . . .
[X] **UA** Ukraine . . . . . .
[X] **UG** Uganda . . . . . .
[X] **US** United States of America . . . . . .
[X] **UZ** Uzbekistan . . . . . .
[X] **VN** Viet Nam . . . . . .

Check-boxes reserved for designation States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

[ ] . . . . . .
[ ] . . . . . .
[ ] . . . . . .
[ ] . . . . . .

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designations(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI **PRIORITY CLAIM** [ ] Further pri[redacted]laims are indicated in the Supplemental Box

The priority of the following earlier app[redacted]n(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) US	17 April 1996 (17.04.96)	60/015,699	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

[X] The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1)

Box No. VII **INTERNATIONAL SEARCHING AUTHORITY**

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA/ EP

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII **CHECK LIST**

This international application contains the following number of sheets:

1. request: 3 sheets
2. description: 44 sheets
3. claims: 11 sheets
4. abstract: 1 sheets
5. Drawings: 3 sheets

Total :62 sheets

This international application is accompanied by the item(s) marked below:

1. [ ] separate signed power of attorney
2. [ ] copy of general power of attorney
3. [ ] statement explaining lack of signature
4. [ ] priority document(s) *identified in Box No. VI as item(s):*
5. [X] fee calculation sheet
6. [ ] separate indications concerning deposited microorganisms
7. [ ] nucleotide and/or amino acid sequence listing (diskette)
8. [ ] other *(specify)*

Figure No. NONE of the drawings (if any) should accompany the abstract when it is published.

Box No. IX **SIGNATURE OF APPLICANT OR AGENT**

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Agent: Beth E. Arnold

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	[ ] received:
4. Date of timely receipt of the required corrections under PCT Article 11(2):	[ ] not received:
5. International Searching Authority specified by the applicant: ISA/	6. [ ] Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International application No.

Applicant's or agent's file reference: HKA-009.25

Date stamp of the receiving Office

Applicant: Hubert Köster

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE ..... 230.00 T

2. SEARCH FEE ..... S

International search to be carried out by _____
(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee
The international application contains _____ sheets.

first 30 sheets ..... 590.00 b_1

_____ x 12.00 = b_2
remaining sheets additional amount

Add amounts entered at b_1 and b_2 and enter total at B ..... B

Designation Fees
The international application contains _____ designations.

_____ x $143.00 = D
number of designation fees payable *(maximum 11)* amount of designation fee

Add amounts entered at B and D and enter total at I I
(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT ($15.00 per patent or applicition) P

5. TOTAL FEES PAYABLE
Add amounts entered at T, S, I and P, and enter total in the TOTAL box .....
TOTAL

[ ] The designation fees are not paid at this time.

MODE OF PAYMENT

[ ] authorization to charge deposit account (see below)	[ ] bank draft	[ ] coupons
[ ] cheque	[ ] cash	[ ] other *(specify)*:
[ ] postal money order	[ ] revenue stamps	

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/_____

[ ] is hereby authorized to charge the total fees indicated above to my deposit account.
[ ] is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.
[ ] is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

06-1448 _____ _____
Deposit Account Number Date *(day/month/year)* Signature

PATENT COOPERATION TREATY

DOCKETED
DUE: 10-16-98
Supp IDS if nec.

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

ARNOLD, Beth E.
FOLEY, HOAG & ELIOT LLP
One Post Office Square
Boston, MA 02109-2170
ETATS-UNIS D'AMERIQUE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	16.07.98
Applicant's or agent's file reference HKA-009.25	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US97/06509	17/04/1997	17/04/1996

Applicant
KOESTER Hubert et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

RECEIVED
JUL 23 1998
F.H. & E. LLP
PATENT DEPT.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	DA ROCHA, O. Tel. (+49-89) 2399-8101

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
HKA-009.25		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US97/06509	17/04/1997	17/04/1996
International Patent Classification (IPC) or national classification and IPC		
C07H19/06		
Applicant		
KOESTER Hubert et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 55 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/11/1997	16. 07. 98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Korsner, S-E Telephone No. (+49-89) 2399-8554

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US97/06509

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-6,10,12,16,16a, 17,18,20,22,24-27, 30,38-40,42	as received on	30/04/1998	with letter of	27/04/1998
6a,7-9,11,13-15, 19,21,23,28,29,31-37, 41,43,44	as received on	01/07/1998	with letter of	29/06/1998

Claims, No.:

2 (part),4,6-13, 18,19,20 (part), 32-36	as received on	30/04/1998	with letter of	27/04/1998
1,2 (part),14-17, 20 (part),21-31	as received on	01/07/1998	with letter of	29/06/1998

Drawings, sheets:

1/3-3/3	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☒ the claims, Nos.: 3, 5, 25
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-2, 4, 6-24, 26-36
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-2, 4, 6-24, 26-36 (see, however, below)
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-2, 4, 6-24, 26-36
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

V. Reasoned statement

Note that Claims 3, 5 and 25 have been withdrawn by the Applicant during the international phase.

- - -

Novelty (Article 33(2) PCT)

The claimed process and compositions are novel in view of the cited prior art.

Inventive step (Article 33(3) PCT)

Inventive step can be acknowledged for the core of the invention as disclosed in the Description.

It is, however, considered that the present scope has been expanded over the factual disclosure in the Description.

For instance, Claim 1 refers broadly to any chemical process for preparing immobilized molecules whereby at least three different protecting groups are used.
The use of terms such as "moieties", "regioselective manner", "uniquely derivatized", "unique substituents" admits no clear interpretation unless further definitions are given.
Reference is made to page 8 and pages 23-28 for final consideration in a later national or regional phase.

VIII. Certain observations

A number of amendments were made by the Applicant during the international phase.
Some of them have been accepted; the following are still to be settled with a view to the original application documents:

Claim 17: the definitions of R1, R3
Claim 32: "n is an integer from 1 -100" and the definitions of R1, R3

Page 10: the addition of the originally missing lines after REACTION on line 15 until line 23 (inclusive).

Notes
a) Claim 13 has been amended to recite "...silicon wafers..." with the reply of 29/06/98, but no amended sheet has been submitted.
b) There is no definition for "n" on page 7, compound 3.

Final remark. A further prior art document has been mentioned during the international examination:
Solid Phase Synthesis & Combinatorial Libraries
Fourth International Symposium
12th - 16th September, 1995
Edingburgh, Scottland
Mayflower Scientific Limited, Birmingham, 1996 (Editor: Roger Epton)
Weiler et al; "High-loaded supports and the NPE/NPEOC-strategy: An efficient combination for large scale synthesis of oligonucleotides", pages 239-242.

(Oral disclosure in 1995, therefore no prior art under Rule 64 1(a) PCT)

Certificate of Express Mail

I hereby certify that the foregoing Patent Application is being deposited with the United States Postal Service as Express Mail, Postage Prepaid, "Post Office to Addressee," in an Envelope Addressed to: Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231 on this date of October 19, 1998.

Lisa A. Fletcher

NAME: Lisa A. Fletcher
Express Mail Label: EM529003205US
Date of Deposit: October 19, 1998

Applicant: KOSTER, et al.
Attorney Docket Number: HKA-009.01
Title: A COMBINATORIAL PROTECTING GROUP STRATEGY FOR MULTIFUNCTIONAL MOLECULES

PA T COOPERATION TRE

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
HKA-009.25		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 97/06509	17/04/1997	17/04/1996
Applicant		
HUBERT, Koster		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. -
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/06509

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H19/06 C07H19/10 C07H19/16 C07H19/20 C07H21/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TETRAHEDRON, vol. 51, no. 19, 1995, OXFORD GB, pages 5557-5562, XP002042680 LEIKAUF, ECKART ET AL: "A new colorimetric protecting group allowing deprotection under neutral conditions" see page 5557 ---	39
P,X	TETRAHEDRON, vol. 52, no. 20, May 1996, pages 6913-6930, XP002042681 DUMONTET, VINCENT ET AL: "A combinatorial protecting group strategy for oligonucleotide synthesis" see the whole document --- -/--	1-42

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search
6 October 1997

Date of mailing of the international search report
20 -10- 1997

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer
Day, G

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ACTA CHEMICA SCANDINAVICA SER. B, vol. 37, no. 9, 1983, pages 857-862, XP002042682 HEIKKILA, JARMO ET AL: "The 2-nitrophenylsulfenyl (Nps) group for the protection of amino functions of cytidine, adenosine, guanosine and their 2'-deoxysugar derivatives" see page 859 ---	41,42
X	TETRAHEDRON LETTERS, vol. 31, no. 18, 1990, OXFORD GB, pages 2549-2552, XP002042683 STENGELE K.-P. AND PFLEIDERER W.: "IMPROVED SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES" see page 2550 ---	41,42
A	WO 92 02533 A (CHIRON CORP., USA) 20 February 1992 see the whole document ---	1,25
X	WO 95 03315 A (OXFORD GLYCOSYSTEMS LTD) 2 February 1995 see the whole document ---	1-8, 25-31
X	WO 96 03424 A (THE SCRIPPS RESEARCH INSTITUTE) 8 February 1996 see page 10 - page 11; claims -----	1

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/06509

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9202533 A	20-02-92	US 5430138 A	04-07-95
		AT 150760 T	15-04-97
		CA 2088255 A	28-01-92
		DE 69125380 D	30-04-97
		DE 69125380 T	28-08-97
		EP 0543906 A	02-06-93
		JP 5509317 T	22-12-93
WO 9503315 A	02-02-95	AU 7193494 A	20-02-95
WO 9603424 A	08-02-96	AU 3235195 A	22-02-96
		CA 2195089 A	08-02-96
		EP 0773953 A	21-05-97

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
HKA-009.25		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US97/06509	17/04/1997	17/04/1996
International Patent Classification (IPC) or national classification and IPC		
C07H19/06		
Applicant		
KOESTER Hubert et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 55 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/11/1997	16. 07. 98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Korsner, S-E Telephone No. (+49-89) 2399-8554

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US97/06509

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-6,10,12,16,16a, 17,18,20,22,24-27, 30,38-40,42	as received on	30/04/1998	with letter of	27/04/1998
6a,7-9,11,13-15, 19,21,23,28,29,31-37, 41,43,44	as received on	01/07/1998	with letter of	29/06/1998

Claims, No.:

2 (part),4,6-13, 18,19,20 (part), 32-36	as received on	30/04/1998	with letter of	27/04/1998
1,2 (part),14-17, 20 (part),21-31	as received on	01/07/1998	with letter of	29/06/1998

Drawings, sheets:

1/3-3/3	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☒ the claims, Nos.: 3, 5, 25
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive st p or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-2, 4, 6-24, 26-36
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-2, 4, 6-24, 26-36 (see, however, below)
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-2, 4, 6-24, 26-36
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

V. Reasoned statement

Note that Claims 3, 5 and 25 have been withdrawn by the Applicant during the international phase.

- - -

Novelty (Article 33(2) PCT)

The claimed process and compositions are novel in view of the cited prior art.

Inventive step (Article 33(3) PCT)

Inventive step can be acknowledged for the core of the invention as disclosed in the Description.

It is, however, considered that the present scope has been expanded over the factual disclosure in the Description.

For instance, Claim 1 refers broadly to any chemical process for preparing immobilized molecules whereby at least three different protecting groups are used.
The use of terms such as "moieties", "regioselective manner", "uniquely derivatized", "unique substituents" admits no clear interpretation unless further definitions are given.
Reference is made to page 8 and pages 23-28 for final consideration in a later national or regional phase.

VIII. Certain observations

A number of amendments were made by the Applicant during the international phase.
Some of them have been accepted; the following are still to be settled with a view to the original application documents:

Claim 17: the definitions of R1, R3
Claim 32: "n is an integer from 1 -100" and the definitions of R1, R3

Page 10: the addition of the originally missing lines after REACTION on line 15 until line 23 (inclusive).

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a) Claim 13 has been amended to recite "...silicon wafers..." with the reply of 29/06/98, but no amended sheet has been submitted.
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Weiler et al; "High-loaded supports and the NPE/NPEOC-strategy: An efficient combination for large scale synthesis of oligonucleotides", pages 239-242.

(Oral disclosure in 1995, therefore no prior art under Rule 64 1(a) PCT)

A Combinatorial Protecting Group Strategy for Multifunctional Molecules

1. Background of the Invention

Traditionally, drug development has not been based on genetic information. More rational approaches are currently possible, however, based on accumulated knowledge regarding the molecular mechanisms of infectious particles (viruses, bacteria, yeast, fungi and protozoa) and the target sites for antibiotics on the molecular level. Two new approaches which have promise for a more rational drug design are combinatorial chemistry (Gordon, E.M., et al., *J. Med. Chem.*, **1994**, 37, 1385-1401; Alper, J., *Science*, **1994**, 264, 1399-1401) and antisense (Cohen, J.S., et al., *Scientific American international edition*, December **1994**, pages 50-55).

In combinatorial chemistry, a large number of all variants of a specific family of compounds is synthesized and investigated for specific affinity to targeted molecules i.e. receptor binding sites. The antisense approach utilizes suitably modified oligonucleotide sequences, which are designed to bind to essential regions for gene expression or virus or cellular replication resulting in complete suppression of the encoded functions.

H-phosphonate or phosphoamidite chemistries employing solid phase methods in automated DNA synthesizers are most efficient for the synthesis of oligonucleotides. The phosphoamidite method using β-cyanoethyl phosphoamidites as reactive nucleotide building blocks is the most prevalent synthesis method due to the quantitative condensation yields despite an oxidation step in every cycle (Sinha, N.D. et

al., *Tetrahedron Lett.*, **1983**, 24, 5843-46; Sinha, N.D. et al., *Nucleic Acids Res.*, **1984**, 12, 4539-57; Froehler, B.C. et al., *Nucleic Acids Res.*, **1984**, 14, 5399-5407; Froehler, B.C. and Matteuci, M.D., *Tetrahedron Lett.*, **1986**, 27, 469-72; Garegg, P.J. et al., *Tetrahedron Lett.*, **1986**, 27, 4051-54; Sonveaux, E., *Bioorg. Chem.*, **1986**, 14, 274-325; Uhlmann, E. and Peyman, A., *Chem. Rev.*, **1990**, 90, 543-84).

According to this method, DNA is synthesized typically in the 3'-5'-direction by using temporary acid labile 4, 4'-dimethoxytrityl (DMTr) groups. The base (acyl amide bonds) and phosphate protection (β-cyanoethyl - deprotected via β-elimination) and the ester linkage to the support are cleaved in a single step by a nonselective reaction with concentrated aqueous ammonia.

To be useful as drugs, oligonucleotides must be able to penetrate through cell walls and nuclear membranes without undergoing enzymatic degradation. Unmodified oligonucleotides are generally unsuitable for this purpose. Therefore the development of modified oligonucleotides is essential for the antisense/triplex DNA approach. Various modifications have been introduced which mainly alter the internucleotide bond (i.e. methyl phosphonates, phosphorothioates and -dithioates, phosphate triesters, phosphoamidates, replacement of the internucleotide bond involving non-phosphorus containing moieties such as PNAs), the base, 2'-deoxyribose or linkage of various molecules at the 3'- or 5'-OH end of the oligonucleotide (Uhlmann, E. and Peyman, A., *Chem Rev.*, **1990**, 90, 543-84; Nielsen, P. et al., *Science*, **1991**, 254, 1497; Beaucage, S.L. and Iyer, R.P., *Tetrahedron.*, **1993**, 49, 6123; Nielsen, P. et al., *Nucleic Acids Res.*, **1994**, 22, 703 -10).

Standard synthetic procedures typically result in depurination by the removal of the DMTr group in each elongation cycle (Shabarova, Z., Bogdanov, A. in

Advanced Organic Chemistry of Nucleic Acids, VCH Verlagsgesellschaft Weinheim, Germany, **1994**). In addition, since the synthesis usually is 3'-5'-directed, oligonucleotides substituted at their 3'-OH end are not easily available. Further, nonselective deprotection by ammonia is disadvantageous for the synthesis of modified DNA, if protecting groups are part of the modification strategy of oligonucleotides.

Antisense/ triplex oligonucleotides have special requirements. The hybridization must be specific and strong enough to guarantee a sufficient blocking of mRNA or nuclear DNA target sequences. In addition, the oligonucleotides should be modified to protect against enzymatic degradation (e.g. by exo- and endonucleases) and to facilitate the passage through the cytoplasmic membrane (to access mRNA sequences) and the nuclear membrane (to target DNA sequences by forming triple helices). To be of therapeutic value, obviously, the modified oligonucleotides must also be non-toxic and the synthetic process must be amenable to easy and cost-effective upscaling.

Whether the target sequences of mRNAs are available for hybridization (i.e. located in loops or single stranded areas and not hidden in stem or tertiary structures) cannot be predicted with absolute certainty (Engels, J., *Natur. Chem. Techn. Lab.*, **1991**, 39, 1250-54). In addition, a synthetic DNA may also bind to unexpected targets such as proteins (Cohen, J.S. et al., *Scientific American, international edition*, December **1994**, pages 50-55) as observed in tissue culture treated with phosphorothioate oligonucleotides. This could lead to further requirements and fine-tuning for the modification, since improvements in one aspect may cause a disadvantage in another. For example the introduction of polycyclic aromatic compounds can lead to higher affinity for the complementary strand due to the intercalating properties but at the same time reduced specificity could result in an increased toxicity or mutagenicity

(Engels, J., *Nachr. Chem. Techn. Lab.*, **1991**, 39, 1250-54). In the triple helix approach there is a demand for special structures if the target sequences do not consist of a continuous stretch of purine residues which is a prerequisite for triple helix formation (Cohen, J.S., et al., *Scientific American international edition*, December **1994,** pages 50-55).

Progress in the syntheses of modified oligonucleotides is remarkable but only a few of the requirements can be fulfilled in one synthesis process since all available procedures lack versatility. Solid phase oligonucleotide synthesis using e.g. monomeric phosphodimorpholino amidites permits the creation of a variety of oligonucleotide phosphate triesters (Uznanski, B. et al., *Tetrahedron Lett.*, **1987,** 28, 3401-04). However, the diversity of modification is limited to derivatizations of the phosphate moiety alone. In another example insertion of (R)- and (S)-3',4'-*seco*-thymidine in oligodeoxynucleotides (modification of the 2-deoxyribose) (Nielsen, P., et al., *Nucleic Acids Res.*, **1994,** 22, 703-10) resulted only in oligomers with good hybridization properties and stability against 3'-exonuclease degradation. All current synthetic methodologies and strategies are hampered by limited versatility and flexibility since the introduction of each modification requires a separate oligonucleotide synthesis run. The development of optimized modification schemes is therefore time consuming and costly.

There is a tremendous demand for synthetic strategies and methodologies which allow the generation of an almost unlimited amount of sequence specific modifications which can be obtained from one synthesis run. In addition, generation of combinatorial libraries of the same oligonucleotide sequence in various states of protection and/or modification allows the selection of molecules with affinities to non-nucleic acid molecules such as receptor sites by using the oligonucleotide backbone as an

oligomeric scaffold exhibiting different patterns of functionalities available for specific molecular recognition processes.

Summary of the Invention

The problems discussed above, which can be overcome by this invention, can be summarized as follows:

1) Using new protection schemes and solid phase synthesis, oligonucleotides are obtained in 5' to 3' direction using phosphoamidites and avoiding depurination. The 3'-OH protecting group employed is suitable as a purification handle for HPLC purification and can be detected in the visible spectral region with high sensitivity to determine condensation yields.

2) Each protecting group of the oligonucleotide (inclusive of the support-spacer moiety at the 5'- or 3'-OH end of the oligonucleotide) is removable selectively (multiselectivity of deprotection, principle of orthogonality). Furthermore, phosphate and base protecting groups can be removed and selected at preprogrammed positions. By substitution reactions, a simple and rapid synthesis of a variety of derivatives for the antisense/triplex concept can be made available. All possible derivatizations can be performed with only one oligonucleotide synthesis run. This versatile synthesis method is therefore amenable to scale-up.

3) Furthermore, the selective and orthogonal procedures and derivations can be applied to structures other than oligonucleotides. Thereby generating variously modified molecules to be tested for interactions with a specific molecular target or biological recognition process.

The above and further features and advantages of the instant invention will become clearer from the following Detailed Description and Claims.

Brief Description of the Drawings

Figure 1 is an HPLC chromatogram of a mixture of the oligodeoxynucleotide d(TAGCT) obtained by 5'-3' and 3'-5' directed syntheses.

Figure 2 is an HPLC chromatogram of a mixture of the oligodeoxynucleotide d(TTTT) obtained by 5'-3' and 3'-5' directed syntheses.

Figure 3 is a MALDI-TOF mass spectrum of the oligodeoxynucleotide d(TAGCT) obtained by 5'-3' directed synthesis.

Figure 4 is a MALDI-TOF mass spectrum of the oligodeoxynucleotide d(TTTT) obtained by 5'-3' directed synthesis.

Figure 5 is a MALDI-TOF mass spectrum of the oligodeoxynucleotide d(TAGCT) obtained by 3'-5' directed synthesis.

Detailed Description of the Invention

As used herein, the following terms and phrases shall have the following meanings:

"monomer", or "building block" shall refer to a molecule with core structure M and plural reactive moieties that can be selectively protected or functionalized.

"oligomer", "oligomeric compound" or "polymer" refers to more than one covalently linked monomer or building block.

$$_{B/}R^{2A/B} = {}_{B \text{ or } B}R^{2A} \text{ or } {}_{B}R^{2B}$$

$$R^{4A/B} = R^{4A} \text{ or } R^{4B}$$

"npeoc/npe protection" means npeoc protection or npeoc and npe protection as shown in the description of scheme 1 for the npeoc/npe protected bases.

Selective deprotections allow the following 16 deprotection combinations: fully protected oligomer, fully deprotected oligomer (①, ②, ③, ④) or partially deprotected oligomers after the following combinations of deprotection reactions, deprotected at positions: (①, ① + ②, ① + ② + ③, ① + ③, ① + ④, ① + ③ + ④, ① + ② + ④, ②, ③, ④, ② + ③, ② + ④, ③ + ④, ② + ③ + ④). Within the combinations of deprotection reactions, the order of deprotection can be chosen. In addition, the use of differently base and/or phosphorus protected building blocks **1** and **2** (scheme 1) during the oligonucleotide synthesis furnishes sequences with base and/or phosphate specific open functionalities. After each deprotection, the nucleophilic group of the oligomer can be reacted with a new substituent or can remain unprotected. Only a minimal quantity of support is needed to create such a newly derivatized oligomer, so that a large variety of derivatizations is possible with just one oligomer synthesis run. New substituents can be introduced into the immobilized oligonucleotide by the reaction of an excess of the appropriate reagents in their reactive forms (e.g. acyl chlorides, anhydrides), in case of the phosphate group in presence of condensing agents (e.g. aromatic sulfonyl chlorides or derivatives thereof) (Heikkila, J., Balgobin, N., Chattopadhyaya, J. *Acta Chem. Scand.*, **1983**, B37, 857-62; Köster. H. *Nachr. Chem. Techn. Lab.*, **1979**, 27, 694-700). Any excess of the reagent can then be removed, e.g. by filtration. The simplicity of these substitutions can be compared with the "capping"-step in DNA synthesis and in principle can be performed on an automated DNA synthesizer.

npeoc/npe groups is necessary to guarantee sufficient hybridization properties of the derivatizated oligomers with complementary nucleic acid sequences.

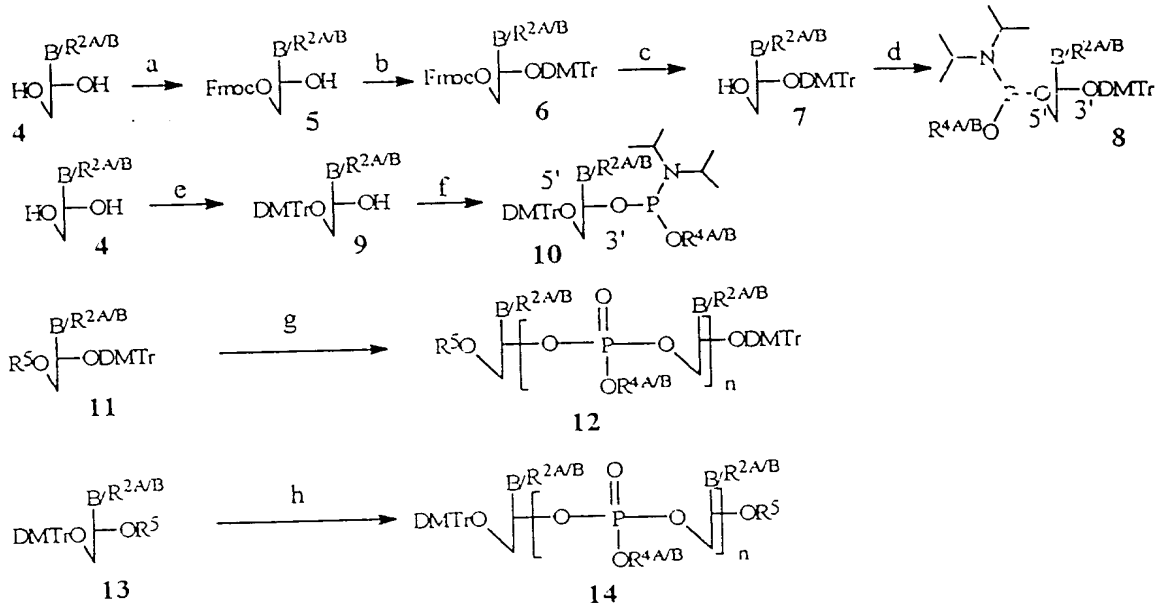
Base protection:

The npeoc/npe protection was found to be stable during deprotection conditions of compound **3** at position ① - ④ (scheme 1 and 2) with the reagents I -IV (table 1). During the npeoc/npe deprotection with DBU reagent, no removal of the new substituents at ① - ④ is desired. These new substituents are linked for example at positions ① and ④ *via* (trityl) ether or carbonic acid ester bonds e.g., at ② *via* phosphate ester bonds e.g., at ③ *via* amide bonds to the oligomer. The stability of phosphate ester, carbonic acid ester and the nucleoside base amide bond during npeoc/npe deprotection has been described (Stengele, K.P., Pfleiderer, W., Tetrahedron Lett., **1990**, 31, 2549-52; Himmelsbach, F., Schulz, B.S., Trichtinger, T., Ramamurthy, C., Pfleiderer, W., Tetrahedron, **1984**, 40, 59-72). These deprotection conditions were found not to affect the trityl ether bond and the nps base protection. Other base protecting groups, in addition to the npeoc/npe protection, should be suitable for use in the process of the invention.

Phosphate protection:

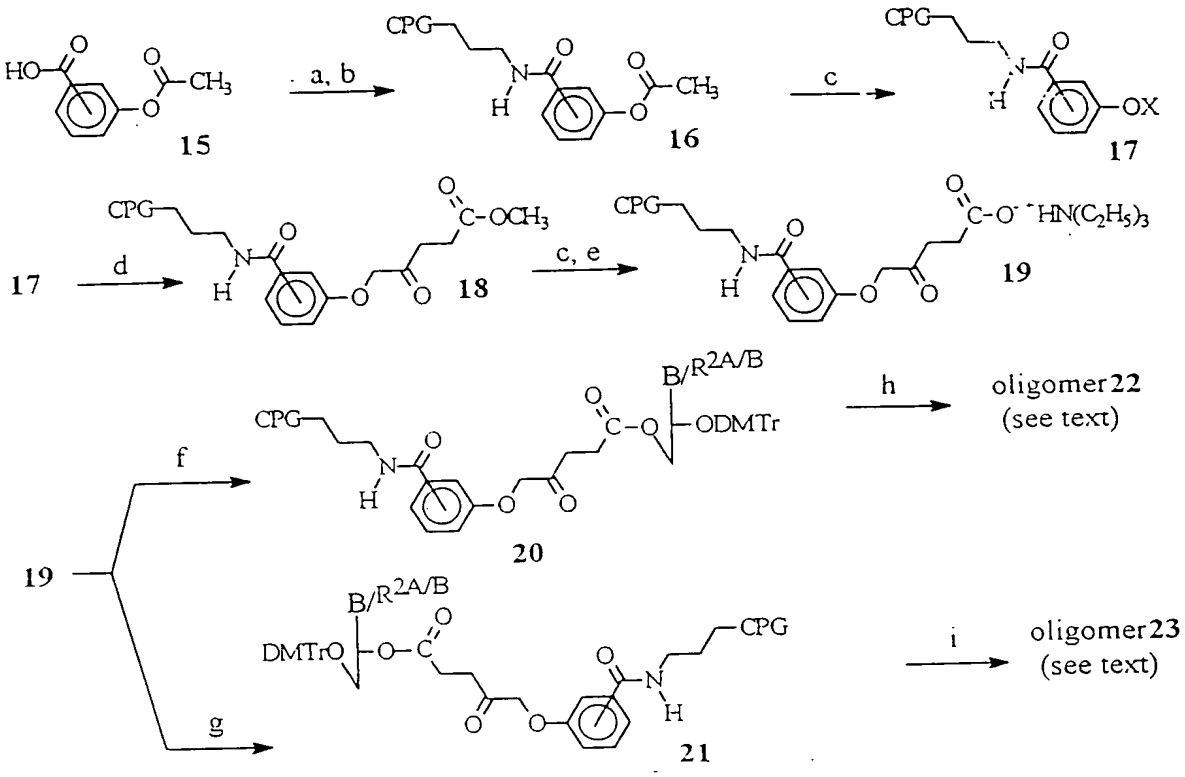
The stability of R^{4B} during the deprotections at ① , ③ and ④ is not absolutely necessary. If R^{4B} is removed, e.g. during deprotection at position ④ using reagent IV, reaction of the OH group at position ④ with an acyl chloride would result in a mixed

Scheme 3



a: selective 5'-OH protection. b: tritylation with DMTr chloride. c: 5'-OH deprotection, e.g. with *tert*-butyl amine reagent II (table 1) or e.g. with triethyl amine reagent. d: phosphitylation. e: selective 5'-OH protection with DMTr chloride. f: phosphitylation. g: 5'-3' directed oligonucleotide synthesis with **11** and **8**. h: 3'-5' directed oligonucleotide synthesis with **13** and **10** R^5: CPG-----$CH_2COCH_2CH_2CO$-(support anchored levulinyl group); n, B, $B/^{R2A/B}$, $R^{4A/B}$: see Scheme 1.

Scheme 4

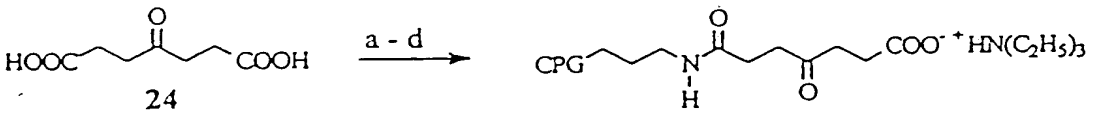

HO
O
O
CH3
15
a, b
CPG
N
H
O
O
CH3
16
c
CPG
N
H
O
OX
17
17
d
CPG
N
H
O
C-OCH3
O
O
18
c, e
CPG
N
H
O
C-O⁻ HN(C2H5)3
O
O
19
19
f
CPG
N
H
O
B/R2A/B
C-O
ODMTr
O
O
20
h
oligomer22
(see text)
19
g
B/R2A/B
DMTrO
O-C
O
CPG
N
H
O
O
O
21
i
oligomer23
(see text)

Description of Scheme 4:

aromatic substitution: ortho, meta or para a: formation of acid anhydride with DCC. b: aminolysis of the formed anhydride with aminopropyl CPG. c: saponification with 32% ammonia solution ($X = NH_4^+$) or K_2CO_3/ methanol/water (ratio: 6.95 g/87.4 ml/69.5 ml; $X = K^+$)[1]). d: reaction with 5-bromo-levulinic acid methyl ester in DMF[2]). e: protonation with 2% $KHSO_4$ solution, deprotonation with triethyl amine. f,g: reaction with compound 7 or 9 (scheme 3) respectively in ethylene dichloride e.g. with DCC[3]) capping of unreacted carboxyl functionalities with methanol (Gupta, K.C. et al., *Nucleic Acids Res.*, **1991**, 19, 3019-25). h, i: oligonucleotide synthesis with amidite **8** or **10** (scheme 3) respectively to the oligomers **22** or **23** (fully protected oligomers).

Remarks: 1) Washing to remove alkaline solution 2) washing to remove NH_4Br or KBr, other washing steps are not listed 3) or reaction of the imidazolide of compound **19** e.g. with compound 7 or 9 (scheme 3).

Scheme 5

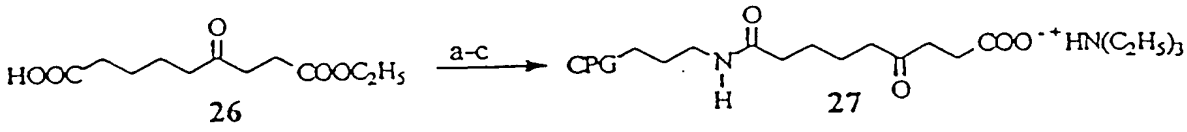


24 25

a: formation of acid anhydride of **24**; aminolysis with e.g.: 2.2mmol of compound **24**, 0.43 mmol DCC, 0.02 mmol 4-dimethylaminopyridine in 4 ml dioxane/triethyl amine 9/3 (v/v) and 1 g aminopropyl CPG; capping reaction with O.33 ml acetic anhydride; washing with dioxane, methanol, DMF and water. b: reaction with K_2CO_3/ methanol/ water (ratio: see scheme 4), washing with water. c: protonation with 2% $KHSO_4$ solution, washing with

water, ethanol and ether. d: reaction with triethyl amine to compound 25, washing with ethylene dichloride. Further steps correspond to the steps f-i of scheme 4.

Scheme 6



26 27

a: reaction of **26** (Johnson, W.S. and Hunt, R.H., *J Amer. Chem. Soc.*, **1950**, 72, 935-39) with DCC e.g. to the acid anhydride. b: aminolysis with aminopropyl CPG. c: saponification, protonation with 2% $KHSO_4$ solution, deprotonation with triethyl amine. Further steps correspond to the steps f-i of scheme 4.

Based on the schemes presented above, one of skill in the art can modify the schemes to accommodate phosphotriester and other suitable methods to generate a combinatorial set of protected molecules having a plurality of moieties, wherein each moiety can be individually deprotected and subsequently derivatized. It is intended that all such modifications fall within the scope of the instant invention.

The following findings demonstrate the feasibility of this extension of the synthetic strategy with the levulinic acid ester bridge. The base protection of nucleosides protected with the 2-nitrophenylsulfenyl (nps) group is rather stable with strongly acidic solutions (Heikkila, J., Balgobin, N., Chattopadhyaya, J., *Acta Chem. Scand.* **1983**, B37, 857-62). We found that stability against depurination in 80% acetic acid decreases as follows: 2'deoxy-N^6-nps-adenosine (A_d^{nps}) >> 2'-deoxy-N^2-nps-guanosine (G_d^{nps}) > 2'-

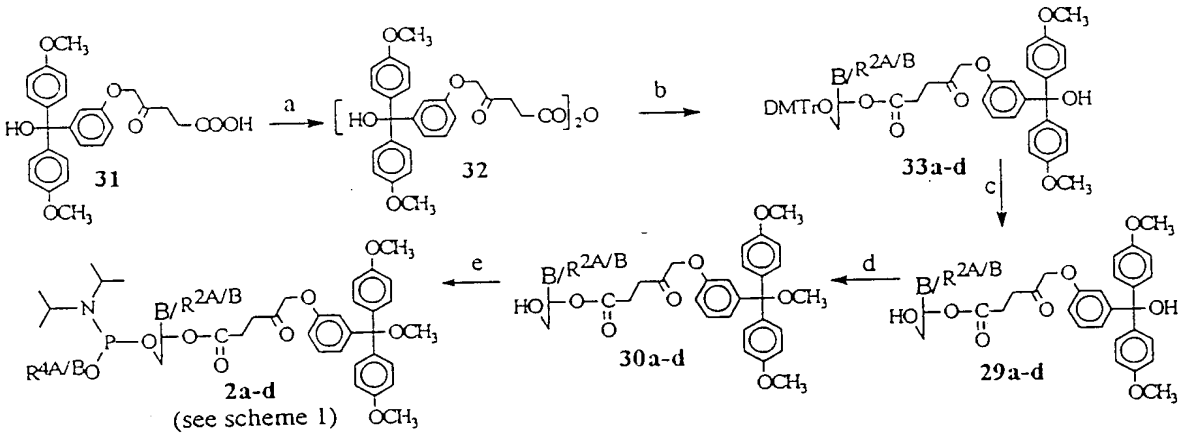
group. Removal of the ß-cyanoethyl group was carried out after synthesis of the protected d(TTTT) with *tert*-butyl amine reagent (table 1), after synthesis of the protected d(TAGCT) with 0.5M DBU in acetonitrile (Stengele, K.P., Pfleiderer, W., *Tetrahedron Lett.*, **1990,** 31, 2549-52) (together with the removal of the base and the 3'-OH protection). Because of the lability of the 3'-OH protection with DBU reagent, the levulinyl group should be substituted by an acyl group stable with DBU reagent before removing the oligomer from the support, if maintaining of the purification handle effect is desired. The 3-{4-[bis-(4-methoxyphenyl)-methyl]-phenyl}-propionyl group of compound **36** (scheme 8), the triphenylmethoxyacetyl (Werstiuk, E.S., Neilson, T., *Can. J. Chem.*, **1972,** 50, 1283-91) or the diphenyl-*tert*-butyl silyl group (Köster, H., Biernat, J, McManus, J., Sinha, N.D., **1985,** *Natural Products Chemistry,* could be such an acyl group. Further experimental steps were similar to the 3'-5' directed DNA synthesis (Sinha, N.D., Biernat, J., Köster, H., *Tetrahedron Lett.*, **1983,** 24, 5843-46; Sinha, N.D., Biernat, J., McManus, J., Köster, H., *Nucleic Acids Res.*, **1984,** 12, 4539-57; Sonveaux, E., *Bioorg. Chem.*, **1986,** 14, 274-325).

To demonstrate identity of the synthesized oligomers following the different synthetic routes (3'-5' versus 5'-3' direction) the oligomers were fully deprotected and analyzed by HPLC. Simultaneous analyses of either the two d(TAGCT) or d(TTTT) oligomers resulted in one single peak, proving identity (Figures 1 and 2). The fully deprotected oligomers d(TAGCT) and d(TTTT) were further characterized by MALDI-TOF mass spectra (figures 3 and 4). In figure 5 a spectrum of d(TAGCT) obtained by the 3'-5' directed synthesis is shown.

G_d^{nps}, A_d^{nps} and C_d^{nps} are not affected by 0.02M iodine reagent which is used for oxidation reaction during oligodeoxynucleotide synthesis. Experiments for base specific deprotections: G_d^{nps}, C_d^{nps} are stable with 0.5M DBU in acetonitrile for 24h, A_d^{nps} only shows a slight deprotection after this time. Compounds **30b-d** were stable with reagents II and III for at least 24h, the stability of the npeoc/ npe base protection with reagents I and IV is demonstrated e.g. by the synthesis of the oligomer d(TAGCT).

The following Scheme 7 shows the synthesis of the building block **2** used for the oligonucleotide synthesis of scheme 1 and 2; and Scheme 8 shows the synthesis of the building block **1** used for the oligonucleotide synthesis of scheme 1 and 2.

Scheme 7

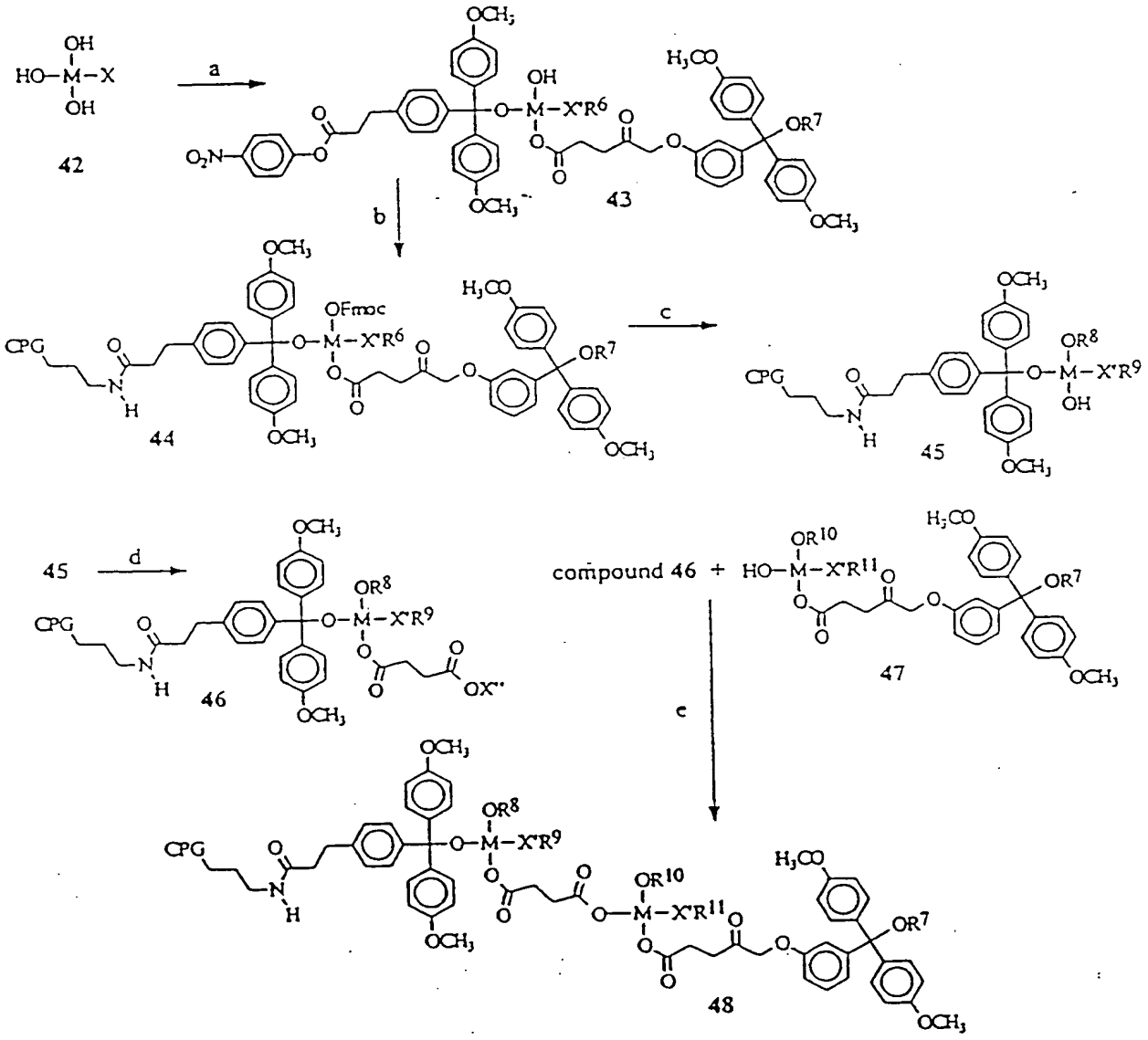


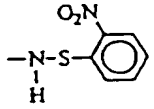
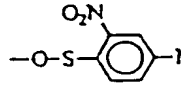
a: condensation of **31** with DCC. b: alcoholysis of **32.** c: detritylation. d: methylation

e: alcoholysis of $[(CH_3)_2CH]_2NP(Cl)OCH_2CH_2CN$ with **30a-d**

Selective and orthogonal deprotections is possible with multifunctional molecules whose core structure M (scheme 9) is different to the structure of oligomer **3** (i.e. low molecular weight multifunctional molecules or biomolecules such as peptides, lipids and oligosaccharides). Use of the process of the invention facilitates creation of a high number of derivatives for combinatorial experiments (Gordon, E.M., Barrett, R.W., Dower, W.J., Fodor, S.P.A., Gallop, M.A., *J. Med. Chem.*, **1994**, 37, 1385-1401; Alper, J., *Science*, **1994**, 264, 1399-1401). Multifunctional molecules need not be limited by the presence of phosphate, OH and amino functionalities (as in oligonucleotides) but could contain only OH functionalities or other combinations of functional groups. The Fmoc group in the 5'-*O*-Fmoc-2'-deoxythymidine (Gioeli, C., Chattopadhyaya, J.B., *J. Chem. Soc. Chem. Commun.*, **1982**, 672-74) showed orthogonal deprotection properties with I-IV (table 1) and the 2,4-dinitrophenylsulfenyl (dnps) group in the dnps ethyl ester (Kharasch, N., McQuarrie, D.P., Buess, C.M., *J. Amer. Chem. Soc.*, **1953**, 75, 2658-60) reveals comparable selective deprotection properties with reagents I-IVa to the 2-nitrophenylsulfenyl (nps) group in the nps amide moiety.

Scheme 9



X : NH_2 or OH, X'R6 :  or  R7 : H or CH_3

X'' : protonated tertiary amine M : core structure

Description of scheme 9:

a: protection of compound **42** with **32** (scheme 7)[1], **39** (scheme 8), 2,4-dinitro- or 2-nitrophenylsulfenyl chloride b: aminolysis with aminopropyl CPG, followed by reaction with Fmoc chloride c:orthogonal deprotections of the Fmoc (9-fluorenylmethoxycarbonyl) group and group R^6 and derivatizations with new substituents R^8 and R^9 respectively, orthogonal deprotection of the levulinic ester moiety d: reaction with succinic anhydride e: reaction of compound **46** with **47**. Compound **47** is (as compound **46**) a derivative of compound **44** but *otherwise derivatized* (with new substituents R^{10} and R^{11} in compound **47** in contrast to R^8 and R^9 in **46**). **47** was removed from the support. The reaction to **48** can be carried out as described by Gupta, K.C. et al., *Nucl. Acids Res.*, **1991**, 19, 3019-25. The succesfull reaction to **48** can be monitored by vis spectroscopy of **48** after treatment with acid.

1) After substitution with compound **32** the tertiary OH functionality can be methylated.

Scheme 9 shows a general way to easily create a high number of derivatives. Compound **43** could be obtained e.g. by successive monosubstitutions using a substantial excess of compound **42** and its products respectively with **39** (scheme 8), **32** (scheme 7), 2,4-dinitrophenylsulfenyl (dnps) chloride or 2-nitrophenyl sulfenyl (nps) chloride (order not obligatory). An excess of **42** can apparently be avoided if regioselective reagents such as **39** are employed. If **42** has an amino function (X = NH_2), transient protection of the OH groups e.g. with trimethylsilyl chloride followed by

protection of the amino function with nps chloride are the first steps. A great excess of **42** should not be necessary in this case.

The structure of the protecting groups is very useful for the reaction control by thin layer chromatography (tlc) during the synthesis of compound **43**. Each reaction step can be controlled by a specific colorimetric effect and UV-detection. This is demonstrated by the following description. If a compound **42** with e.g. four hydroxyl groups is monosubstituted by compound **32** (scheme 7), treatment with acid leads to an orange product (trityl cation), but the colorimetric trityl moiety is not cleaved. After the second monosubstitution with **39** (scheme 8), detection with acid leads to two orange products, because one of the trityl moieties is now cleaved off. Additionally, intensive yellow colour can be observed by ammonia vapour (or by primary and secondary amines), due to released p-nitrophenolate ions. The product obtained after the third monosubstitution with dnps chloride already shows yellow colour without any detection reagent (and of course the other colorimetric effects). Protection of the last free hydroxyl group with Fmoc chloride should be done *after* the reaction of compound **43** with aminopropyl CPG, because of the sensitivity of Fmoc esters in the presence of amino groups. Nevertheless, the last free hydroxyl group of a sample of compound **43** can be substituted by a nucleoside derivative (the reactive form of 5'-*O*-DMTr-T_d-*O*3'-succinic mono ester e.g.). By contact with sugar spray reagent and heating with a fan an *additional* green coloured product can be observed on tlc (due to the superposition of the blue colour of the nucleoside and the orange colour of the trityl moieties). This shows the possibility to control four successive monosubstitions by different colorimetric effects.

Example 1: 3'-O-levulinyl esters of the nucleosides 30a-d (building block 2)

Compound **32** was prepared *in situ* by reacting levulinic acid derivate **31** (Leikauf, E., Köster. H., Tetrahedron, **1995**, 51, 5557-62) (3.78 g, 8.39 mmol) with N,N'-dicyclohexylcarbodiimide (1.80 g, 8.74 mmol) in dry dioxane (25 ml). N,N'-dicyclohexylurea was removed by filtration and washed with dioxane. The solution was divided in four equal parts and the solvents were evaporated *in vacuo*. To each of the four residues of anhydride **32** was added one of the four following protected nucleosides: 5'-*O*-DMTr-2'-deoxythymidine, 5'-*O*-DMTr-N^4npeoc-2'-deoxycytidine, 5'-*O*-DMTr-N^6-npeoc-2'-deoxyadenosine, 5'-*O*-DMTr-N^2-npeoc-O^6npe-2'-deoxyguanosine (1.00 mmol of each; base protected deoxynucleosides were from Chemogen, Konstanz) (Stengele, K.P., Pfleiderer, W., Tetrahedron Lett., **1990**, 31, 2549-52) and 4-dimethylaminopyridine (0.0100 g, 0.0819 mmol) in 1.64 ml pyridine. Completion of reaction was checked by thin layer chromatography. 30 min after the addition of a mixture of 0.130 ml of glacial acetic acid and 0.245 ml pyridine, 0.046 ml water were added, 60 min later an excess of ethyl acetate was added, the N,N'-dicyclohexylurea removed by filtration and washed with ethyl acetate. The mixture was extracted with water, 5% aqueous sodium hydrogen carbonate and water. After drying with sodium sulfate, the solvent was evaporated, then co-evaporated with toluene. The residues were directly detritylated with 80% acetic acid and the reaction was monitored by thin layer chromatography. The solutions were poured into an excess of water (about 10fold) and the aqueous mixtures were extracted with ethyl acetate. The organic phase was washed with 5% aqueous sodium hydrogen carbonate and water. After drying, the solvent was evaporated, then co-evaporated with toluene (to remove remaining acetic acid). The residues were directly methylated by adding to each a solution of 200 ml methanol and 1 ml glacial acetic acid. If there were some insoluble

bath until a clear solution was obtained. Another 9.95 g (41.6 mmol) lead dioxide was added and dissolved. The reaction was monitored by thin layer chromatography (dichloromethane/ methanol 99/1, v/v). During the reaction a side product appeared which traveled between the educt **37** and product **38**. The reaction was terminated when UV intensity of the residual **37** spot equaled the side product. The reaction mixture was poured on ice/ water (3 1) and extracted with dichloromethane. The organic layer was extracted with water and dried with Na_2SO_4. Solvents were evaporated and some remaining acetic acid removed by co-evaporation with toluene. The raw product (29.8 g) was dissolved in dichloromethane and purified by silica gel 60H (Merck, Darmstadt; No.7736, 1200 g) column chromatography; elution was carried out in the presence of 0.03% pyridine using a step gradient from dichloromethane to dichloromethane / ethanol 99/1, v/v). Fractions containing compound **38** were combined and the solvents evaporated. The sirupous residue gradually crystallized under a petroleum ether layer after rubbing with a glass rod. Yield: 15.1 g (50%). 2.15 g (7%) of the starting material **37** was recovered. - *^1H NMR* (250 MHz, $CDCl_3$):δ = 2.70 (s, 1H, R_3C-O<u>H</u>), 2.94 (t, 2H. -C<u>H</u>$_2$-CH_2-), 3.1 (t, 2H, -CH_2-C<u>H</u>$_2$-), 3.78 (s, 6H, -OC<u>H</u>$_3$), 7.28-6.78 (m, 14H, aryl-<u>H</u>), 8.21 (d, 2H, O_2N-aryl-<u>H</u>, ortho). - *^{13}C NMR* (63 MHz, $CDCl_3$):δ = 30.37 (t, -<u>C</u>H$_2$-CH_2-), 35.8 (t, -CH_2-<u>C</u>H$_2$-), 55.26 (q, aryl-O<u>C</u>H$_3$), 81.27 (s, R_3<u>C</u>-OH), 113.22, 122.41, 125.17, 127.87, 128.08, 129.08, (d, <u>C</u>-H, aryl), 138.35, 139.41, 145.32, 145.85, 155.32 (s, aryl, quarternary), 158.7 (s, R_2<u>C</u>-OCH$_3$, aryl), 170.45 (s, -<u>C</u>OOR). - *MS* (EI): m/z (rel. intensity): m/z calculated for $C_{30}H_{27}NO_7$ (M^+): 513; found: 513 (22), 496 (9, M - OH^+), 406 (12, M - C_6H_4-OCH_3^+), 243 (93, M - O_2N-C_6H_4-OOC-CH_2-CH_2-$C_6H_4^+$), 135 (100). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{30}H_{27}NO_7$ (M^+): 513; found: 513 (19), 496 (90, M - OH^+), 406 (16, M - C_6H_4-OCH_3^+), 391 (11, M - O_2N-$C_6H_4^+$), 375 (3, M - O_2N-C_6H_4-O^+), 243 (52, M - O_2N-C_6H_4-OOC-CH_2-CH_2-$C_6H_4^+$), 135 (100). - *Elementary Analysis* (%): Found: C, 70.47/ 70.78; H, 5.35/ 5.38; N, 2.75/ 2.74; $C_{30}H_{27}NO_7$ requires C, 70.16; H, 5.3; N, 2.73.

Example 7: p-Nitrophenyl-3-{4-[bis-(4-methoxyphenyl)-chlormethyl]-phenyl}-propionate (39) (building block 1)

Compound **38** (0.600 g, 1.17 mmol) was refluxed in acetyl chloride (6 ml) for 3 h. Solvents were evaporated and remaining traces of acetic acid or/ and acetyl chloride removed by co-evaporation with toluene. The residue was directly converted to compound **40**.

Example 8: Alcoholysis of compound 39 with 30a to compound 40 (building block 1)

The sirupous raw product of compound **39** (max. 1.17 mmol) was dissolved in dry pyridine (4.1 ml) and compound **30a** (0.450 g, 0.653 mmol) was added. After 19 h another 0.140 g (0.203) mmol) **30a** was added. 24 h later monitoring by thin layer chromatography indicated no further conversion. Pyridine (3 ml) and ethanol (0.3 ml) were added and the solution poured into an excess of water after 5 min. The water solution was extracted with ethyl acetate. The organic phase was extracted with water and dried with Na_2SO_4- Solvents were evaporated and the residue azeotropically dried with toluene. The raw product (1.38 g) was dissolved in dichloromethane and purified by silica gel 60 (Merck, Darmstadt; No. 9385, 80 g) column chromatography; elution was carried out in presence of 0.1% pyridine with dichloromethane/ ethanol 99/1, v/v). Fractions containing **40** were combined and the solvents evaporated. The residue was dissolved in dichloromethane (11 ml) and precipitated into hexane (220 ml). Yield: 0.468g (46%). - *^1H NMR* (400 MHz, $CDCl_3$ and a trace of [D_5]pyridine):δ = 1.36 (s, 3H, -C$\underline{H}_3$ of thymine), 2.5-2.4 (m, 2H,H2'^{a}/H2'^{b}), 2.63 (t, 2H, -C$\underline{H}_2$-CH_2-, 3'-OH protecting group), 2.9 (t, 2H, -CH_2-C$\underline{H}_2$-, 3'-OH protecting group), 2.9 (t, 2H, -C$\underline{H}_2$-CH_2-, 5'-OH protecting group), 3.04 (t, 2H, -CH_2-C$\underline{H}_2$-, 5'-OH protecting group), 3.04 (s, 3H, R_3C-OC$\underline{H}_3$), 3.45 (m, 2H, H5''^{a}/H5''^{b}), 3.77 (s,12H, aryl-OC$\underline{H}_3$), 4.14 (m, 1H, H4'), 4.54 (s, 2H, -CO-C$\underline{H}_2$-O-), 5.47 (m,1H, H3'), 6.43 (t, 1H, H1'), 7.37-6.7 (m, 26H, aryl-$\underline{H}$), 7.6 (s, 1H, H6), 8.21 (d, 2H, O_2N-aryl-$\underline{H}$, ortho), 8.9 (s, 1H, N-$\underline{H}$ of thymine). - *^{13}C NMR* (101 MHz, $CDCl_3$ and a trace of [D_5]pyridine):δ = 11.66 (q, -$\underline{C}H_3$ of thymine), 27.4 (t, -$\underline{C}H_2$-CH_2-, 3'-OH protecting group), 30.26 (t, $\underline{C}H_2$-CH_2-, 5'-OH protecting group), 33.77 (t, -CH_2-$\underline{C}H_2$-, 3'-OH protecting group), 35.68 (t, -CH_2-$\underline{C}H_2$-, 5'-OH protecting group), 37.88 (t, C2'), 51.90 (q, R_3C-O$\underline{C}H_3$), 55.21, 55.27 (q, aryl-O$\underline{C}H_3$, 3'-OH and 5'-OH

protecting group, position not defined) 63.74 (t, C5'), 72.74 (t, -CO-$\underline{C}H_2$-O-), 75.69 (d, C3'), 83.96 (d, C4'), 84.38 (d, C1'), 86.29, 87.05 (s, $R_3\underline{C}$-OCH_2-, 5'-OH protecting group, $R_3\underline{C}$-OCH_3, 3'-OH protecting group, position not defined), 111.49 (s, C5 of thymine), 112.29, 113.09, 113.37, 114.59, 121.81, 122.37, 128.07, 128.38, 128.93, 130.08, 130.18 (d, $\underline{C}$-H, aryl), 125.17 (d, $\underline{C}$-H, O_2N-aryl, ortho), 135.61 (d, C6 of thymine), 135.06, 135.4, 138.55, 142.89, 145.33, 147.57, 155.28 (s, aryl, quarternary), 150.37 (C2 of thymine), 157.3, 158.51 (s, $R_2\underline{C}$-OCH_3, and s, $R_2\underline{C}$-O-CH_2-CO-: 3'-OH protecting group, aryl, position not defined), 158.84 (s, $R_2\underline{C}$-OCH_3, aryl, 5'-OH protecting group), 163.57 (s, C4 of thymine), 170.35 (s, -$\underline{C}$OOR, 5'-OH protecting group), 172.07 (s, -$\underline{C}$OOR, 3'-OH protecting group), 205.94 (s, -$\underline{C}$O-). - *$^1H^1H$* and *$^1H^{13}C$ 2D NMR* spectra are determined (data not shown). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{67}H_{65}N_3O_{17}$ (M^+): 1183; found: 1183 (4), 1152 (35, M - OCH_3^-), 1137 (2, M - NO_2^-), 496 (100, fragment M - OH^- of compound **38**). - *Elementary Analysis* (%): Found: C, 67.63/ 67.89; H, 5.63/ 5.69; N, 3.49/ 3.51; $C_{67}H_{65}N_3O_{17}$ requires C, 67.95; H, 5.53; N, 3.55.

Example 9: Aminolysis of compound 40 with aminopropyl CPG to building block 1

Compound **40** (0.160 g, 0.135 mmol) was dissolved in dry dioxane (0.311 ml) and dry pyridine (0.032 ml). A suspension of aminopropyl CPG (0.405 g, CPG-10-500, Biosyntech, Hamburg) in 1.27 ml dry N,N-dimethylformamide and 0.160 ml (0.116 g, 1.15 mmol) dry triethylamine was added and the suspension shaken during 21.5 h. An intensive yellow colour indicated beginning reaction caused by released p-nitrophenolate ions. The suspension was shaken during 21.5 h. A ninhydrin test at this stage indicated the existence of free amino groups on the support. To acylate, "cap", these groups, dry triethylamine (0.030 ml) and acetic anhydride (0.090 ml) were added and the suspension was shaken for another 60 min. After this time a negative ninhydrin test was obtained. The support was washed successively with N,N-dimethylformamide, ethanol, dioxane, ether (100 ml each) and dried *in vacuo*. Analysis for the extent of 3'-OH protected nucleoside attached to the support was done spectrophotometrically. An accurately

5) *Capping reagent 1*: N-methylimidazole/ pyridine/ acetonitrile, 12/ 1O/ 78, v/v, GEN 905027; *capping reagent 2*: acetic anhydride/ acetonitrile, 12/ 88, v/v, GEN 905026. The tetrazole, oxidation and the capping reagents are purchased from PerSeptive Biosystems GmbH, Hamburg.

6) *Hydrazine reagent*: 0.5M hydrazine reagent IVb (table 1).Reagent of high quality have to be used: bidestilled water, acetic acid p.a. (Merck, Darmstadt No. 63), hydrazinium hydrate (Merck, Darmstadt No. 804608), pyridine p.a. (Merck, Darmstadt No. 7463).

7) *TCA reagent*: 40% trichloroacetic acid in dichloromethane (percentage by weight).

8) Amidite and tetrazole solutions and acetonitrile (DNA grade) to dissolve amidites and to carry out the last washing in step 2 (table 2) before the condensation were kept under molecular sieve 0.3 nm, freshly activated in a microwave oven, stored under argon and taken or added by syringes *via* septa.

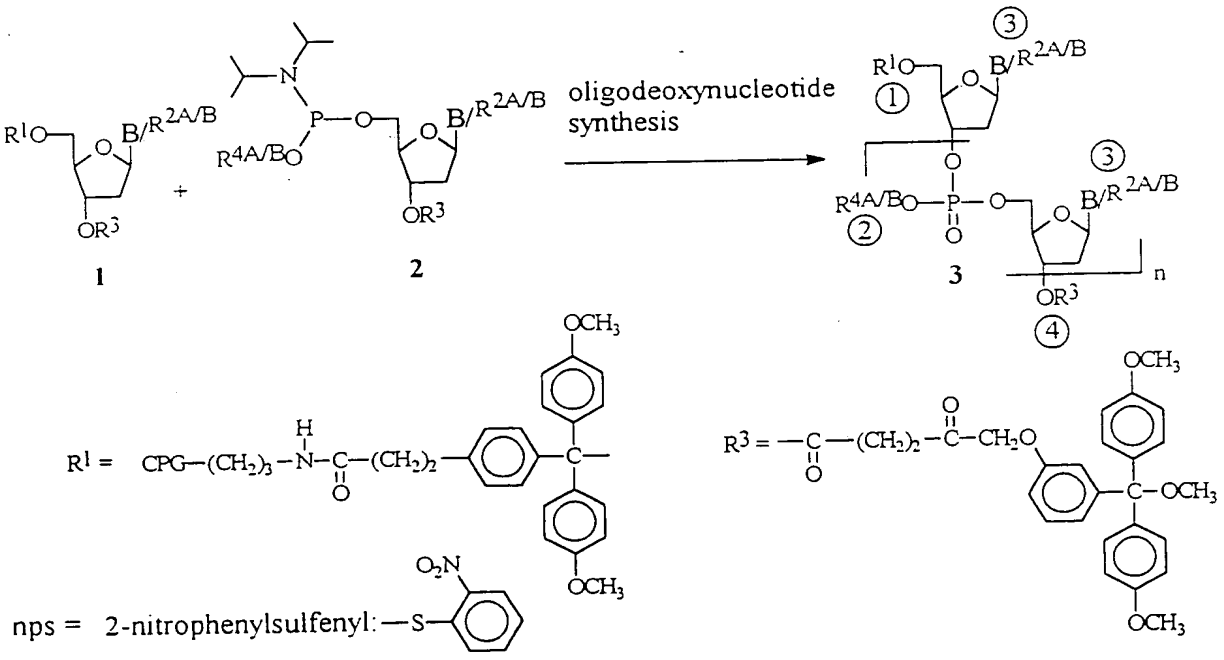
9) Acetonitrile for washing steps only had to be "HPLC grade", except for the last washing before the condensation.

"Sequence specific derivations" means derivations at defined bases and/or phosphate moieties due to the different kind of base and phosphate protection group (R^{2A} or R^{2B} and R^{4A} or R^{4B}, respectively).

In general, the instant invention features new strategies for chemical polymer synthesis, which permit multiselective deprotection (for example via sequence dependent preprogrammed selection of appropriate nucleotide building blocks) to create polymers with predetermined modifications and/or functionalities. Various combinations of these specifically modified/functionalized monomers or oligomers can generate a combinatorial set of molecules available for specific molecular interaction or recognition experiments.

The following scheme 1 presents a generalized 5' to 3' directed oligonucleotide synthesis resulting in oligomers for sequence specific selective and orthogonal deprotections and for subsequent derivatizations. The first step in the oligonucleotide synthesis cycle is the removal of R^3.

Scheme 1



$$_{B/}R^{2A/B} = {}_{B \text{ or } B}R^{2A} \text{ or } {}_{B}R^{2B}$$

$$_{R}^{4A/B} = R^{4A} \text{ or } R^{4B}$$

$$R^{2A/B} = R^{2A} \text{ or } R^{2B}$$

R^{4A} = β-cyanoethyl as protecting group for selective and orthogonal deprotection with reagent II (table 1),

R^{4B} = a protecting group stable with reagent II (table 1).

B = a natural or modified nucleobase, which does not require a protecting group during synthesis,

B^{R2A}, B^{R2B} = a natural or modified nucleobase with a protecting group,

R2A R2B = different protecting groups, e.g. R2A is the nps protecting group in: *N*4-nps-cytosine (Cnps), *N*6-nps-adenine (Anps), *N*2-nps-guanine (Gnps) for selective and orthogonal deprotection according to table 1 and e.g. R2B is the npeoc,npe protection in: *N*4-npeoc-cytosine (Cnpeoc), *N*6-npeoc-adenine (Anpeoc), *N*2-npeoc-*O*6-npe-guanine (Gnpeoc,npe) stable under the deprotection conditions of table 1, npeoc = 2-(4-nitrophenyl)-ethoxycarbonyl, npe = 2-(4-nitrophenyl)-ethyl.

n: number of condensation reactions; ①, ②, ③, ④: protective positions; CPG: Controlled-Pore-Glass.

As shown in scheme 1, a 2'-deoxyoligonucleotide, **3**, is synthesized, e.g. by the phosphoamidite method (Sinha, N.D., Biernat, J., Köster, H. *Tetrahedron Lett.*, **1983**, 24, 5843-46; Sinha, N.D., Biernat, J., McManus, J., Köster, H. *Nucleic Acids Res.*, **1984**, 12, 4539-57; Sonveaux, E. *Bioorg. Chem.*, **1986**, 14, 274-325). However, in contrast to the usual 3' to 5' addition, the synthesis is performed in the 5' to 3' direction using the building blocks **1** and **2**. During an elongation cycle, the temporary protecting group, R^3, is removed, e.g. using a neutral hydrazine reagent IV (table 1) before the condensation step and the acidified filtrate of the hydrazinolysis solution is spectrophotometrically measured to determine the preceding condensation yield. In this manner, a trityl assay as typically used with the 4, 4'-dimethoxytrityl group, is possible. In addition, there is little risk of depurination, since acidic conditions are not used during the synthesis cycles.

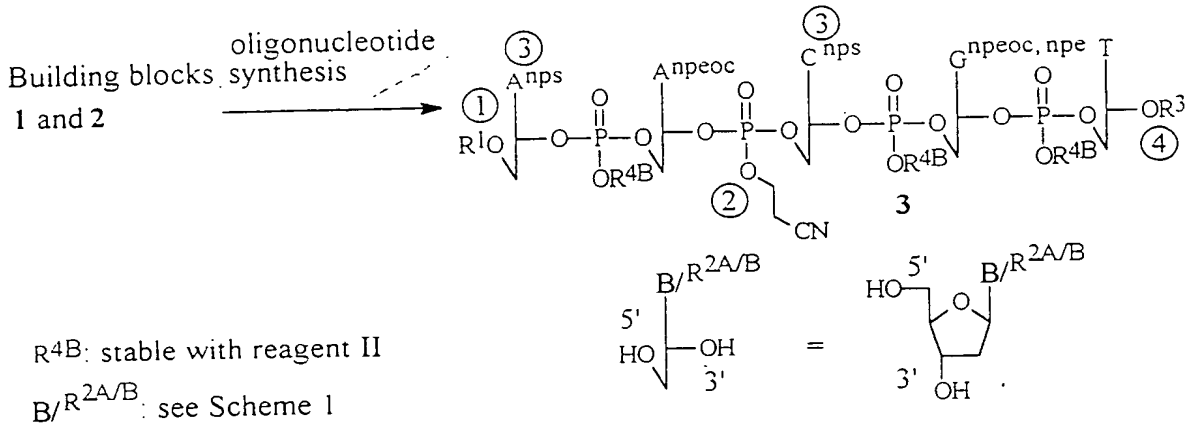
Selective and orthogonal deprotections are possible if at the linkages ①-④ of oligomer **3**, deprotections are selectively done as shown in the following table 1.

Table 1. Selective and orthogonal deprotection at oligomer 3.

Deprotection at linkage in 3	Reaction	Deprotection reagent
①	detritylation	I: 80% acetic acid
②	decyanoethylation	II: *tert*butyl amine/ pyridine 1/9 (v/v)
③	base deprotection	III: p-thiocresole in pyridine/DMF 3/7 (v/v): 3mmol/ml
④	hydrazinolysis	IVa: 1M hydrazinium hydrate in pyridine/ glacial acetic acid/ water (4:3:0.35, v/v), pH 5.4
		IVb: 0.5M hydrazinium hydrate in pyridine/ glacial acetic acid/ water (4:1:0.25, v/v), pH 6.5

Scheme 2 shows an example of an immobilized fully protected oligomer **3** for sequence specific derivatizations by the use of differently base and/ or phosphorus protected building blocks **1** and **2** of scheme **1** using the reagents of table 1.

Scheme 2



The selective and orthogonal deprotections and the derivatizations by introducing new substituents can be carried out at positions ① - ④, at ② and ③ in a *sequence specific* way. During the derivatizations at ① - ④ only the npeoc/npe base protection remains intact. In contrast, the phosphate protecting group R^{4B} needs to remain intact if derivatizations at ② are to be performed. The npeoc/npe and R^{4B} protecting groups only serve to carry out *sequence specific* derivatizations at ② and/or ③. *After* the derivatizations at least the bases, protected with npeoc/npe groups have to be deprotected without removing new substituents at ① - ④ at the same time. The removal of the

anhydride at the phosphate moiety, which subsequently could be either transformed to a newly protected function or hydrolyzed to the phosphodiester. Of course, the substitution at position ② should be carried out before; R^{4B} must be stable with reagent II, to guarantee a sequence specific derivatization at position ②.

The phosphate protection with the p-chlorophenyl group e.g. is stable with reagent II in contrast to the β-cyanoethyl group (Hsiung, H.M., *Tetrahedron Lett.*, **1982,** 23, 5119-22). The phosphate protection with the o-chlorophenyl group e.g. is stable with 0.5M hydrazine reagent (Watkins, B.E., Kiely, J. S., Rapoport, H., *J.Am. Chem. Soc.*, **1982,** 104, 5702-08). The phosphate protection with the 2,5-dichlorophenyl group e.g. is stable with strong acids as p-toluenesulfonic acid in methylene chloride/methanol (Himmelsbach, F., Schulz, B.S., Trichtinger. T., Ramamurthy, C., Pfleiderer, W., *Tetrahedron*, **1984,** 40, 59-72). During the deprotection of R^{4B} no removal of the new substituents at ① - ④ is desired. The o-chlorophenyl group e.g. allows deprotection with 4-nitrobenzaldoximate without affecting benzoic acid ester and nps amide bonds (Heikkilä, J., Balgobin, N., Chattopadhyaya, J., *Acta Chem. Scand.*, **1983,** B37, 857-62). Further the o-chlorophenyl group e.g. is easily removable with $(n\text{-butyl})_4NF$ (Reese, C.B., Titmas, R.C., Yau, L., *Tetrahedron Lett.*, **1978,** 2727-30). Under these conditions acetic acid ester, trityl ether bonds and the nucleoside base protection with the acetyl or benzoyl groups remain intact (Ogilvie, K.K., *Can.J.Chem.*, **1973,** 51, 3799-3807).

In addition to the described 16 deprotection combinations at positions ① - ④ selective and orthogonal deprotections at the different nps protected bases (e.g. deprotection at C^{nps} before A^{nps} in oligomer **3,** schemes 1 and 2) could lead to a maximum of 64 deprotection combinations. The rate of base deprotection in nps base protected

nucleosides was found to be significantly influenced by the deprotection reagent (thiocresolate concentration and solvents). The rate of deprotection in 0.02M thiocresolate in pyridine decreases as follows: 2'-deoxy-N^2-nps-guanosine (G_d^{nps})>>2'-deoxy-N^4-nps-cytidine (C_d^{nps}) >>2'-deoxy-N^6-nps-adenosine (A_d^{nps}). It would seem to be difficult to identify reagents leading to a reversion of this order to obtain e.g. nps protected cytosine and guanine in the presence of nps deprotected adenine moieties. But such a deprotection state could be achieved by selective deprotection of the C^{nps} and G^{nps} moieties, followed by reprotecting them with groups, stable with thiocresolate reagent. Finally A^{nps} can be deprotected with this reagent. In yet another approach, this protection scheme can be obtained by using the suitably protected nucleotide building blocks during oligomer synthesis.

Compared to current oligodeoxynucleotide syntheses for use in antisense and triplex DNA therapies (Cohen, J.S., Hogan, M.E., Scientific American, Int. Ed., December **1994**, pages 50-5514; Uhlmann, E., Peyman, A., *Chem. Rev.*, **1990**, 90, 543-84; Beaucage, S.L., Iyer, r.P. *Tetrahedron*, **1993,** 49, 6123-94), the new strategy shows a remarkable advantage. All possible derivitizations can be performed with only *one* oligonucleotide synthesis run.

The strategy presented above, can be modified according to other oligonucleotide synthesis schemes. For example, in addition to the phosphoramidite method shown in scheme 1, the strategy can be employed with the phosphotriester and other suitable methods of oligonucleotide synthesis. For the phosphotriester method, chloro substituted phenyl groups and the ß-cyanoethyl group were successfully used as phosphate protection groups (Amarnath, V., Broom, A. D.. *Chem. Rev.* **1977**, 77, 183-217; Reese, C. B., *Tetrahedron*, **1978,** 34, 3143-79). The levulinic acid ester and the

npeoc/npe base protection are stable during the reaction conditions of the phosphotriester method (Himmelsbach, F., Schulz, B.S., Trichtinger, T., Ramamurthy, C., Pfleiderer, W., *Tetrahedron*, **1984,** 40, 59-72; van Boom, J.H., Burgers, P.M.J., *Tetrahedron Lett.*, **1976,** 4875-78). The nps base protection has been successfully used during the oligonucleotide synthesis by the phosphotriester approach (Heikkila, J., Balgobin, N., Chattopadhyaya. J., *Acad Chem. Scand.*, **1983,** B37, 857-62). The structure of oligomers obtained in this way of synthesis is the same as for the oligomer **3** generated by the phosphoamidite method (scheme 1).

In addition, if the oligomer (e.g. **3** in scheme 1) is connected at its 3'-OH or 5'-OH group to the CPG via a levulinic acid ester bridge (cleavable with neutral hydrazine reagent IV) instead of the trityl ether bridge in **3,** a simplified 3'-5' as well as 5'-3' directed DNA syntheses would be available, keeping the advantage of multiselective deprotections, with the trityl moiety for easy detection and as a "purification handle" (Sinha, N.D., Biernat, J., Köster, H. *Tetrahedron Lett.*, **1983**, 24, 5843-46; Sinha, N.D., Biernat, J., McManus, J., Köster, H. *Nucleic Acids Res.*, **1984,** 12, 4539-57; Sonveaux, E. *Bioorg. Chem.*, **1986,** 14, 274-325). For syntheses by the phosphoamidite method, amidites, whose 5'-OH or 3'-OH groups respectively are protected with the 4,4'-dimethoxytrityl (DMTr) group, are used. Scheme 3 shows a general view and scheme 4 to 6 show specific examples.

deoxy-N^2isobutyryl-guanosine (G_d^{ib}) >> 2'-deoxy-N^6-benzoyl-adenosine (A_d^{bz}); G_d^{ib} and A_d^{bz} are exposed to strong acids in every elongation cycle in the standard DNA synthesis process (Sinha, N.D., Biernat, J., Köster, H., *Tetrahedron Lett.*, **1983,** 24, 5843-46; Sinha, N.D., Biernat, J., McManus, J., Köster, H., *Nucleic Acids Res.*, **1984,** 12, 4539-57; Sonveaux, E., *Bioorg. Chem.*, **1986** 14, 274-325). In accordance with Heikkilä, J. et al. (*Acta Chem.Scand.*, **1983,** B37, 857-62), A_d^{nps} does not depurinate with 80% acetic acid, although the main depurination problem in standard DNA synthesis is caused by the A_d^{bz} units. 2'-Deoxy-N^4-nps-cytidine (C_d^{nps}) is stable with 80% acetic acid. No problem caused by depurination is observed with npeoc/ npe base protection (Stengele, K.P., Pfleiderer, W., *Tetrahedron Lett.*, **1990,** 31, 2549-52).

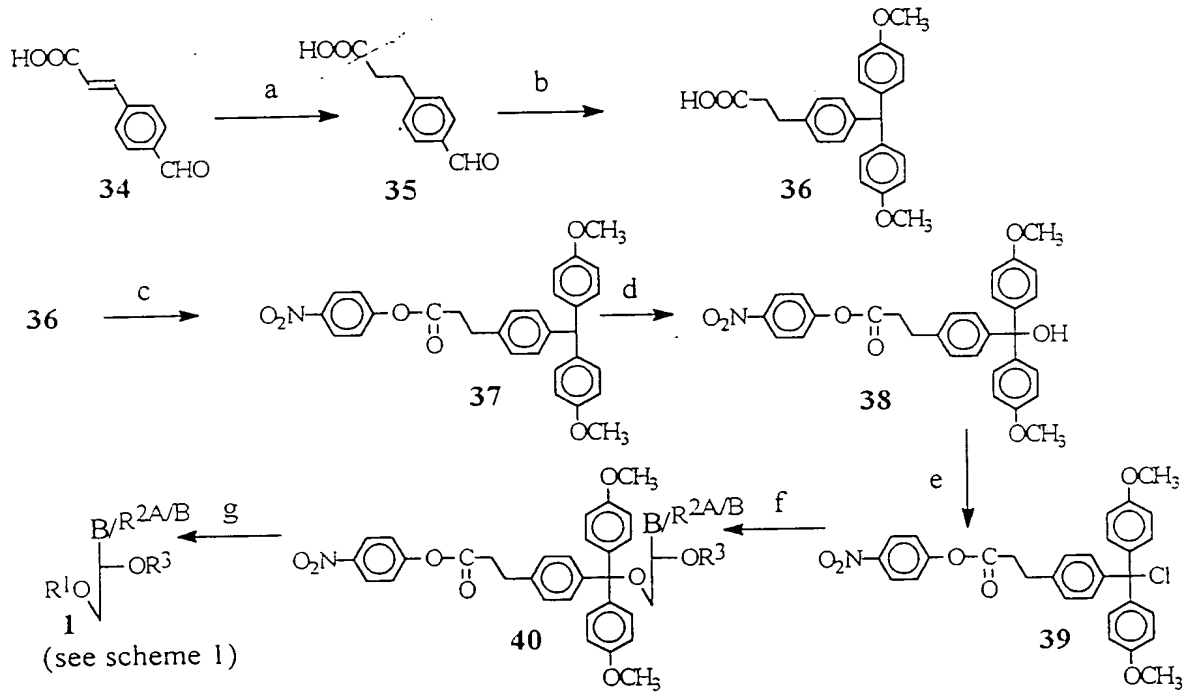
To test the new strategy d(TAGCT) and d(TTTT) were synthesized by a 5'-3' directed DNA synthesis with support **1** (scheme 1, ${}_{B/}R^{2A/B}$ = thymine) and amidites **2** (scheme 1, ${}_{B/}R^{2A/B}$ = thymine, npeoc/ npe protected bases, $R^{4A/B}$ =ß-cyanoethyl, corresponding to **2a - d** of scheme 7). The 3'-OH protection was removed with hydrazine reagent IV (table 1) at near neutral pH, forming a heterocyclic compound which is detected in the visible spectral region with high sensitivity after being acidified (Leikauf, E., Köster, H., *Tetrahedron*, **1995,** 51, 5557-62. To obtain the desired high condensation yields, addition of the amidite solution had to be preceded by the activation with tetrazole. The oligomer could be removed from the support by a short treatment with 80% acetic acid without affecting the 3'-OH protection. Suitability of the 3'-OH protection group as "purification handle" (Sinha, N.D., Biernat, J., Köster, H., *Tetrahedron Lett.*, **1983,** 24, 5843-46; Sinha, N.D. Biernat, J., McManus, J., Köster, H., *Nucleic Acids Res.*, **1984,** 12, 4539-57; Sonveaux, E., *Bioorg. Chem.*, **1986.** 14, 274-325) is comparable with the DMTr

The immobilized fully protected d(TTTT) (oligomer **3**, $_{B/}R^{2A/B}$ = thymine, $R^{4A/B}$ = β-cyanoethyl) already exhibited eight different combinations of deprotection with the reagents I, II, IV of table 1. To demonstrate the orthogonal deprotection (16 deprotection combinations) for the fully protected mixed oligomer **3** of scheme 1 (for the bases A, G, C the protecting group $R^{2A/B} = R^{2A}$ = nps, $R^{4A/B} = R^{4A}$ = ß-cyanoethyl) with the optimized deprotection reagents I-IV of table 1, additional deprotection experiments were carried out with the immobilized oligomers 5'-*O*-R^1 -d(TTTT)-3'-*O*-R^3, 5'-*O*-R^1 - d(TAGCT)-3'-*O*-R^3 (R^1, R^3 as in scheme 1) and in solution with the model compounds 5'-*O*-DMTr-2'-deoxythymidine [(DMTr)T_d], **28, 29a, 30a** (scheme 10), G_d^{nps}, A_d^{nps}, C_d^{nps}. The deprotection reagents removed one protecting group quickly, while the other groups were stable under these conditions for at least 24h. This is demonstrated by the following results: 1) reagent I (80% acetic acid): the 3'-O-protected d(TTTT) and d(TAGCT) are removed from the support after 15 minutes by detritylation, in compound **28** only the DMTr group is cleaved, compound **30a** is only transformed to compound **29a** which is stable, the nps groups of the nps protected nucleosides are not removed (only at G_d^{nps} depurination is observed, but slower compared to G_d^{ib}); 2) reagent II (*tert*-butyl amine reagent): decyanoethylation is complete with compound **28** after 40 minutes, (DMTr)T_d, compounds **29a, 30a** and the nps protected nucleosides are stable; 3) reagent III (thiocresolate reagent): nps groups are removed at G_d^{nps}, C_d^{nps} after 5 minutes, at A_d^{nps} after 45 - 60 minutes, compounds **28, 29a, 30a** are stable; 4) reagents IVa, IVb (hydrazine reagents): delevulination is complete with compounds **29a** and **30a** after 8 minutes, compound **28** and the nps protected nucleosides are stable.

$R^{4A/B}$ = β-cyanoethyl

compounds 33, 29, 30 and 2	$_{B/}R^{2A/B}$
a	thymine
b	N^4-npeoc-cytosine
c	N^6-npeoc-adenine
d	N^2-npeoc-O^6-npe-guanine

Scheme 8

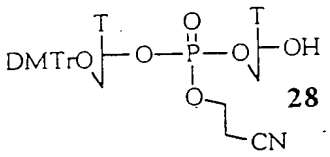


a: hydrogenation. b: electrophilic aromatic substitution with anisole. c: esterification. d: oxidation. e: chlorination of **38**. f. alcoholysis with **30a** (scheme 7, $_{B/}R^{2A/B}$: thymine). g: aminolysis with aminopropyl CPG to compound 1 (R^1, R^3: see Scheme 1).

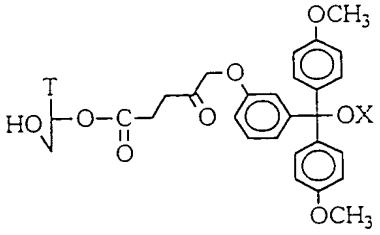
Another embodiment of the invention employs the combinatorial protecting group strategy with other multifunctional molecules. The oligomer **3** of scheme 1 has four protected moieties, suitable for selective and orthogonal deprotection with the reagents I-IV (table 1):1) the ß-cyanoethyl protected phosphate moiety, 2) the tritylether moiety, 3) nps protected amino groups and 4) the levulinic acid ester moiety.

Generalized, the introduction of **39** (scheme 8), **32** (scheme 7) and 2,4-dinitrophenylsulfenyl (dnps) chloride or 2-nitrophenylsulfenyl (nps) chloride and the selective/ orthogonal deprotections of the corresponding protecting groups in a chosen multifunctional molecule can be controlled by different colorimetric effects in each step.

Scheme 10



28



29a X = H

30a X = CH_3

T : thymine

The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, published patent applications and co-pending patent applications, as cited throughout this application are hereby expressly incorporated by reference.

EXAMPLES

Materials and Methods

^1H (400 and 250 MHz) and ^{13}C (101 and 63 MHz) NMR spectra were recorded on a Bruker AMX 400 and a AC 250-P instrument. Samples were dissolved in the presence of tetramethylsilane as internal standard, unless otherwise stated. ^{31}P NMR sprectra were recordered on a Varian Gemini 200BB instrument. External standard: 85% phosphoric acid in the solvent used for the sample (δ = 0.00 ppm), Chemical shifts are given in ppm. Mass spectra were obtained on a Finnigan MAT 311A mass spectrometer

under EI conditions, a VG Analytical 70-250S mass spectrometer under FAB conditions (matrix: 3-nitro-benzyl alcohol, xenon bombardment) and a Finnigan MAT Vision 2000 mass spectrometer under MALDI-TOF conditions (matrix solution: 0.7 mol/1 3-hydroxy picolinic acid and 0.07 mol/ 1 ammonium citrate in acetonitrile/ water, 1/1, v/v). Elementary analyses were performed by the analytical department of the Institute of Organic Chemistry, University of Hamburg. Thin layer chromatography (tlc) was carried out on 60 PF_{254} silica gel coated alumina sheets (Merck, Darmstadt, No 5562). Trityl and sugar containing compounds are visualized with sugar spray reagent (0.5 ml 4-methoxybenzaldehyde, 9 ml ethanol, 0.5 ml concentrated sulfuric acid and 0.1 ml glacial acetic acid) by heating with a fan or on a hot plate. p-Nitrophenyl ester containing compounds are visualized by ammonia vapour. Column chromatography was performed using silica gel from Merck. HPLC results were obtained on a Waters chromatography systems 625 LC with a photodiodearray detector 996 and using reversed phase columns (Waters Nova-Pak C18™, 60, 4μm particles, 3.9 x 300mm, software: Millenium 2.0, eluants were: 0.1 M triethylammonium acetate at pH 7.0 (A) and acetonitrile (B); the column was equilibrated at 30°C at 1ml per min, with 95% A/ 5% B, v/v, with elution using a linear gradient from 5% to 40% B in 40 min, monitored at 254 nm). Spectrophotometric measurements in the UV/ Vis region were performed on a Beckman UV35 and a LKB Ultrospec Plus UV/ Vis spectrophotometer. Solvents were dried and purified before use according to standard procedures. Extractions were monitored by tlc to optimize completion of extraction.

material, it was dissolved in 5-10 ml dichloromethane and a mixture of 100 ml methanol and 0.5 ml glacial acetic acid was added. Monitoring by thin layer chromatography indicated completion of the reaction. The solvents were evaporated under reduced pressure, followed by co-evaporation with toluene (2-3 times). The residues of **30a-d** were purified by silica gel column chromatography (**30a**: silica gel 60H, No. 7736, **30b-d**: silica gel 60°A, No. 9385; Merck, Darmstadt). Silica gel used per gram raw product: **30a**: 25 g, **30b**: 51 g, **30c**: 65 g, **30d**: 51 g; using a step gradient from dichloromethane to dichloromethane/ methanol 98/2 (v/v), in the presence of 0.1% pyridine. Pure fractions were pooled, the solvents removed by evaporation, the residues dissolved in dichloromethane (15 ml per gram residue) and the solutions precipitated into hexane (315 ml per gram residue). Yields: **30a**: 68%, **30b**: 63%, **30c**: 62%, **30d**: 52%.

Compound 30a: *^{1}HNMR* (400 MHz, $CDCl_3$): δ = 1.88 (s, 3H, -C<u>H</u>$_3$ of thymine), 2.5-2.34 (m, 2H, H2'^{a}/H2'^{b}), 2.64 (t, 2H, -C<u>H</u>$_2$-CH$_2$-), 2.93 (t, 2H, -CH$_2$-C<u>H</u>$_2$-), 3.04 (s, 3H, R_3 C-OC<u>H</u>$_3$), 3.8 (s, 6H, aryl-OC<u>H</u>$_3$), 3.9 (m, 2H, H5'^{a}/H5'^{b}), 4.1 (m, 1H, H4'), 4.57 (s, 2H, -CO-C<u>H</u>$_2$-O-), 5.38 (m,1H, H3'), 6.26 (t, 1H, H1'), 7.34-6.7 (m, 12H, aryl-<u>H</u>), 7.55 (s, 1H, H6), 8.93 (s, 1H, N-<u>H</u> of thymine). - *^{13}C NMR* (101 MHz, $CDCl_3$): δ = 12.51 (q, -<u>C</u>H$_3$ of thymine), 27.43 (t,-<u>C</u>H$_2$-CH$_2$-), 33.79 (t, -CH$_2$-<u>C</u>H$_2$-), 37.27 (t,C2'), 51.92 (q, R_3C-O<u>C</u>H$_3$), 55.23 (q, aryl-O<u>C</u>H$_3$), 62.39 (t, C5'), 72.77 (t, -CO-<u>C</u>H$_2$-O-), 75.31 (d, C3'), 85.20 (d, C4'), 85.92 (d, C1'), 86.31 (s, R_3<u>C</u>-OCH$_3$), 111.28 (s, C5 of thymine), 112.37, 113.11, 114.59, 121.83, 128.95, 130.20 (d, <u>C</u>-H, aryl), 135.63, 147.58 (s, R_2<u>C</u>-CR$_2$-OCH$_3$, aryl, quartenary), 136.34 (d, C6 of thymine), 150.52 (s, C2 of thymine), 157.32, 158.52 (s, R_2<u>C</u>-OCH$_3$, and s, R_2<u>C</u>-O-CH$_2$CO-, aryl, position not defined), 163.69 (s, C4 of thymine), 172.26 (s, -<u>C</u>OOR), 206.02 (s, <u>C</u>O-). - *^{1}H^{1}H* and *^{1}H^{13}C 2D NMR*

spectra were determined. - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{37}H_{40}N_2O_{11}$ (M^+): 688; found: 688 (7), 657 (74, M - OCH_3^+), 391 (78), 307 (100). - *Elementary Analysis* (%): Found: C, 64.99/ 64.73; H, 5.98/ 5.82; N, 4.02/ 3.99; $C_{37}H_{40}N_2O_{11}$ requires C, 64.53; H, 5.85; N, 4.07.

Compound 30b:*^{1}HNMR* (400 MHz, $CDCl_3$): δ = 2.38 (m, 1H, H2'^{a}), 2.65 (m, 1H, H2'^{b}, t, 2H, -C<u>H</u>$_2$-CH_2-, 3'-OH protecting group), 2.9 (t, 2H, -CH_2-C<u>H</u>$_2$-, 3'-OH protecting group), 3.04 (s, 3H, R_3C-OC<u>H</u>$_3$), 3.1 (t, 2H, -C<u>H</u>$_2$-CH_2-, base protection), 3.8 (s, 6H, aryl-OC<u>H</u>$_3$), 3.99-3.88 (m, 2H, H5'^{a}/H5'^{b}), 4.19 (m, 1H, H4'), 4.44 (t, 2H, -CH_2-C<u>H</u>$_2$-, base protection), 4.55 (s, 2H, -CO-C<u>H</u>$_2$-O-), 5.38 (m, 1H, H3'), 6.26 (m, 1H, H1'), 7.34-6.7 (m, 13H, aryl-<u>H</u> and H5), 7.38 (d, 2H, O_2N-aryl-<u>H</u>, meta), 8.17 (d, 2H, O_2N-aryl-<u>H</u>, ortho), 8.3-8.2 (s, 1H, N-<u>H</u> and d, 1H, H6 of cytosine).-*^{13}CNMR* (101 MHz, $CDCl_3$): δ =27.43 (t,- C<u>H</u>$_2$-CH_2-,3'-OH protecting group), 33.78 (t, -CH_2-<u>C</u>H$_2$-, 3'-OH protecting group), 34.97 (t, -<u>C</u>H$_2$-CH_2-, base protection), 38.58 (t,C2'), 51.91 (q, R_3C-O<u>C</u>H$_3$), 55.23 (q, aryl-O<u>C</u>H$_3$), 62.12 (t, C5'), 65.49 (t, -CH_2-<u>C</u>H$_2$)-, base protection). 72.76 (t, -CO-<u>C</u>H$_2$-O-), 74.98 (d, C3'), 85.98 (d, C4' and d, C1'), 123.86 (d, <u>C</u>-H. O_2N-aryl, ortho),129.77 (d, <u>C</u>-H, O_2N-aryl, meta), 112.35, 113.1, 114.58, 121.83, 128.95. 130.2 (d, <u>C</u>-H, aryl, d, C5 and d, C6 of cytosine, position not defined),86.31 (s, R_3<u>C</u>-OCH_3), 123.22, 135.64, 147.58, 149.14, 149.40, 149.67 (s, aryl, quarternary, C2, C4 of cytosine and -NH-<u>C</u>O- of the base protection, position not defined), 157.32, 158.52 (s, R_2<u>C</u>OCH_3, and s, R_2<u>C</u>-O-CH_2-CO-, aryl, position not defined), 172.32 (s, -<u>C</u>OOR), 205.97 (s, -<u>C</u>O-). -*^{1} H^{1}H* and *^{1}H^{13}C 2D NMR* spectra were determined. - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{45}H_{46}N_4O_{14}$ (M^+): 866; found: 866 (9), 835 (100, M - OCH_3^+), 307 (87).

Compound 30c: *^{1}HNMR* (400 MHz, $CDCl_3$): δ = 2.48 (m, 1H, H2'^{a}), 2.67 (t, 2H, -C<u>H</u>$_2$-CH_2-, 3'-OH protecting group), 2.96 (t, 2H, -CH_2-C<u>H</u>$_2$-, 3'-OH protecting group), 3.05 (s. 3H, R_3C-OC<u>H</u>$_3$), 3.15 (t, 2H, -C<u>H</u>$_2$-CH_2-, base protection and m, 1H, H2'^{b}) 3.78 (s, 6H, aryl-OC<u>H</u>$_3$), 4.0-3.84 (m, 2H, H5'^{a}/H5'^{b}), 4.29 (m, 1H, H4'), 4.55 (t, 2H, -CH_2-C<u>H</u>$_2$-, base protection and s, 2H, -CO-C<u>H</u>$_2$-O-), 5.58 (m, 1H, H3'), 6.35 (m, 1H, Hl'), 7.34-6.7 (m, 12H, aryl<u>H</u> of DMTr),7.42 (d, 2H, O_2N-aryl-<u>H</u>, meta), 8.08 (s, 1H, H2 or H8 of adenine), 8.14 (d, 2H, O_2N-aryl-<u>H</u>, ortho), 8.73 (s, 1H, H2 or H8 of adenine), 9.04 (-N-<u>H</u> of adenine), - *^{13}CNMR* (101 MHz, $CDCl_3$): δ = 27.49 (t, -<u>C</u>H_2-CH_2-,3'-OH protecting group), 33.86 (t, -<u>C</u>H_2-CH_2-, 3'-OH protecting group), 35.01 (t, -<u>C</u>H_2-CH_2-, base protection), 37.85 (t,C2'), 51.91 (q, R_3C-O<u>C</u>H_3), 55.22 (q, aryl-O<u>C</u>H_3), 63.16 (t, C5'), 65.52 (t, -CH_2-<u>C</u>H_2-, base protection), 72.77 (t, -CO-<u>C</u>H_2-O-), 76.57 (d, C3'), 87.25 (d, C4'), 87.49 (d, Cl'), 123.84 (d, <u>C</u>-H, O_2N-aryl, ortho), 112.32, 113.11, 114.55, 121.87, 128.97, 130.21 (d, <u>C</u>-H, aryl), 129.81 (d, <u>C</u>-H, O_2N-aryl, meta), 86.30 (s, R_3<u>C</u>-OCH_3) 135.57, 145.24, 147.02, 147.72, 149.18, 149.72, 150.04, 150.69 (s, aryl, quartenary, C4-C6 of adenine and -NH-<u>C</u>O- of the base protection, position not defined), 142.31 (d, C2 or C8 of adenine), 152.3 (d, C2 or C8 of adenine), 157.3, 158.54 (s, R_2-<u>C</u>-OCH3, and s, R_2<u>C</u>-O-CH_2-CO-, aryl, position not defined), 171.98 (s, -COOR), 206.14 (s, -<u>C</u>O-). - *^{1}H ^{1}H* and *^{1}H ^{13}C 2D NMR* spectra were determined. - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{46}H_{46}N_6O_{13}$ (M^+): 890; found: 859 (5, M - OCH_3^-), 307 (100). - *Elementary Analysis (%):* Found: C, 62.14/ 62.00; H, 5.26/ 5.17; N, 9.06/ 9.01; $C_{46}H_{46}N_6O_{13}$ requires C, 62.02; H, 5.20; N, 9.43.

Compound 30d:*^{1}HNMR* (400 MHz, $CDCl_3$): δ = 2.44-2.40 (m, 1H, H2'^{a}), 2.67 (t, 2H -C<u>H</u>$_2$-CH_2-, 3'-OH protecting group), 2.96 (t, 2H, -CH_2-C<u>H</u>$_2$-,3'-OH protecting group), 3.04 (s, 3H, R_3C-OC<u>H</u>$_3$), 3.12 (t, 2H, -C<u>H</u>$_2$ -CH_2-, npeoc base

protection and m, 1H, H2'', 3.30 (t, 2H, -C$\underline{H}_2$-CH_2-, npe base protection), 3.8 (s, 6H, aryl-O$C\underline{H}_3$), 3.99-3.82 (m, 2H, H5'/H5''), 4.23 (m, 1H, H4'),4.49 (t, 2H, -CH_2-C$\underline{H}_2$-, npeoc base protection), 4.56 (s, 2H, -CO-C$\underline{H}_2$-O-), 4.82 (t, 2H, -CH_2-C$\underline{H}_2$-, npe base protection) 5.57 (m,1H, H3'), 6.24 (m, 1H, H1'), 7.7-6.7 (m, 16H, aryl-$\underline{H}$ of DMTr, O_2N-aryl-$\underline{H}$, meta and s, 1 H, -N-$\underline{H}$ of guanine), 7.89 (s, 1 H, H8 of guanine). 8.18-8.13 (m, O_2N-aryl-$\underline{H}$, ortho, npe and npeoc group). - *^{13}C NMR* (101 MHz, $CDCl_3$): δ = 27.46 (t,-$\underline{C}H_2$-CH_2-,3'-OH protecting group), 33.86 (t, -CH_2-$\underline{C}H_2$-,3'-OH protecting group), 35.04 (t, -$\underline{C}H_2$-CH_2-, npe and npeoc base protection), 37.37 (t, C2'), 51.92 (q,R_3C-O$\underline{C}H_3$) 55.23 (q, aryl-O$\underline{C}H_3$), 63.01 (t, C5'), 64.97 (t,-CH_2-$\underline{C}H_2$-,npeoc base protection), 67.07 (t,-CH_2-$\underline{C}H_2$-npe base protection), 72.77 (t, -CO-$\underline{C}H_2$-O-), 76.24 (d, C3'), 86.55 (d, C4'), 86.79, (d,C1'), 123.75, 123.82 (d, $\underline{C}$-H, O_2N-aryl, ortho, npe and npeoc base protection, position not defined), 141.33 (d, C8 of guanine), 112.32, 113.11, 114.55, 121.87, 128.96, 129.77, 130.04, 130.21 (d, $\underline{C}$-H, aryl), 86.30 (s, $R_3\underline{C}$-OCH_3,), 135.58, 145.58, 146.94, 147.7, 149.15, 149.42, 149.69, 151.13, 151.47, 152.06, 161.01 (s, aryl, quartenary, C2, C4-C6 of guanine and -NH-$\underline{C}$O of the base protection, position not defined), 157.31, 158.54 (s, $R_2\underline{C}$-OCH_3, and s, $R_2\underline{C}$-O-CH_2-CO-, aryl, position not defined), 172.04 (s, -$\underline{C}$OOR), 206.10 (s, -$\underline{C}$O-). -*^1H ^1H* and *^1H ^{13}C 2D* NMR spectra are determined. -*MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{54}H_{53}N_7O_{16}$ (M^+): 1055; found: 1024 (6, M -OCH_3 $^+$), 307 (100).

Example 2: Phosphoamidites 2a-d (building block 2)

All steps were carried out under inert athmosphere (argon). Organic solvents were free from water and other impurities. Compounds **30a-d** (0.5 mmol of

each) were azeotropically dried with small amounts of pyridine and toluene and dissolved in 2.43 ml ethyl acetate. After the addition of N,N-diisopropyl ethylamine (1.75 mmol, 0.226 g, corresponding to 0.30 ml at room temperature) the reaction flask was capped with a septum and cooled with an ice bath. Chloro-β-cyanoethoxy-N,N-diisopropylaminophosphane (0.610 mmol, 0.144 g, corresponding to 0.117 ml at room temperature, Biosyntech, Hamburg) was added dropwise by a syringe. 15 min later the reaction was allowed to raise to room temperature. Monitoring by thin layer chromatography (about 60 min after starting the reaction) indicated complete conversions to the amidites **2a-d.** The precipitated amine hydrochloride was filtered off using a column type reactor fitted with a sintered glass fritt and washed with 1.5 ml ethyl acetate. The solution was extracted in a separation funnel with cold 5% sodium hydrogen carbonate (2 x 2.8 ml). The organic solution was filtered using the described reactor which contained sodium sulfate, followed by washing of the sodium sulfate layer with ethyl acetate (2 x 1.8 ml). After evaporation of the solvents of the filtrate, a foam was obtained. The amidite was dissolved in 5 ml ethyl acetate (containing 0.1% pyridine) and precipitated into 120 ml of hexane (at -20°C). After filtration using the described reactor the amidite was washed with 12 ml of hexane, dried and stored at -20°C. Yields: **2a:** 86%, **2b**: 72%, **2c**: 78%, **2d**: 80%. - ^{31} *P NMR* (81 MHz, CD_3CN/ CH_3CN, l/l,v/v and a trace of N,N-diisopropyl ethylamine): **2a**:δ = 149.18, 149.35 (diastereomers), **2b:**δ = 149.25, **2c:**δ = 149.07, **2d**:δ = 148.89, 149.16 (diastereomers).

Example 3: 3-(4-Formylphenyl)-propionic acid (35) (building block 1)

Hydrogenation of compound **34** (Pohl, H., *J.prakt.Chem.*, **1934,** 141, 45-60; Skita, A, Ritter,H., *Ber.Dtsch.Chem.Ges.*, **1910**, 43, 3393-99; Paal, C., Harmann, W., *Ber. Dtsch.Chem.Ges.*, **1909**, 42, 3930-39) was carried out in the presence of 5% Pd on activated carbon. - ^{1}*H NMR* (250 MHz, [D_6]DMSO):δ = 2.6 (t, 2H, -C<u>H</u>$_2$,-CH_2-), 2.95 (t, 2H, CH_2-C<u>H</u>$_2$,), 7.45 (d, 2H, <u>H</u>-aryl-CHO, meta), 7.85 (d, 2H, <u>H</u>-aryl-CHO, ortho), 9.96 (s, 1H, -C<u>H</u>O), 12.16(s, 1H, -COO<u>H</u>).

Example 4: 3-{4-[Bis-(4-methoxyphenyl)-methyl]-phenyl}-propionic acid (36) (building block 1)

Compound 35 (25.7 g, 144 mmol) and methoxybenzene (36.8 g, 340 mmol) were stirred in 450 ml glacial acetic acid to dissolve most of the material. The mixture was cooled in an ice bath and immediately concentrated sulfuric acid (225 g, 2290 mmol) added dropwise. The reaction mixture was then stirred at room temperature until thin layer chromatography (dichloromethane/methanol: 8/2, v/v) demonstrated quantitative conversion. The reaction mixture was poured into 3L ice/ water. Subsequently the reaction flask was washed with ether and the ether solution was poured into the ice/water. The orange-white raw product between the aqueous and organic layer was filtrated by suction (if there was still a considerable amount of the raw product under the aqueous and/or dissolved in the ether layer, it was also worked up). The raw product was triturated with 200 ml water, filtrated by suction, again triturated with petroleum ether (bp 60-70°C) and filtrated. It was recrystallized from ether. Yield: 22.3 g (41%). Note: More product **36** can be purified from the crystalline residue of the the mother liquor by silica gel column chromatography or by Soxhlet extraction with petroleum ether (bp 30-50°C). *^{1}H NMR* (250 MHz, $CDCl_3$):δ = 2.65 (t, 2H, -C$\underline{H}_2$-CH_2-), 2.92 (t,2H, -CH_2-C$\underline{H}_2$-), 3.78 (s, 6H, -OC$\underline{H}_3$), 5.40 (s, 1H, R_3C-$\underline{H}$), 7.13-6.77 (m, 12H, aryl-$\underline{H}$). - *^{13}C NMR* (63 MHz, $CDCl_3$, internal standard $CDCl_3$ at 77.00 ppm):δ = 30.1 (t, -$\underline{C}H_2$-CH_2-), 35.48 (t, -CH_2-$\underline{C}H_2$-), 54.8, 55.19 (q, aryl-O$\underline{C}H_3$ and d, $R_3\underline{C}$-H, position not defined), 113.63, 128.1, 129.41, 130.21 (d, $\underline{C}$-H, aryl), 136.46, 137.91, 142.7 (s, aryl, quartenary), 157.93 (s, $R_2\underline{C}$-OCH_3, aryl), 178.85 (s, -$\underline{C}$OOH).- *MS* (EI): m/z (rel. intensity): m/z calculated for $C_{24}H_{24}O_4$ (M^+): 376; found: 376 (100), 345 (9, M-OCH_3 $^+$), 227 (35, M - HOOC-CH_2-CH_2-C_6H_4 $^+$). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{24}H_{24}O_4$ (M^+): 376; found: 376 (48), 345 (8, M-OCH_3 $^+$), 269 (53, M - C_6H_4-OCH_3 $^+$), 227 (38, M - HOOC-CH_2-CH_2-C_6H_4 $^+$). - *Elementary Analysis* (%): Found: C, 76.55/ 76.35; H, 6.71/6.53;$C_{24}H_{24}O_4$ requires C, 76.57; H, 6.43.

Example 5: p-Nitrophenyl-3-{4-[bis-(4-methoxyphenyl)-methyl]-phenyl}-propionate (37) (building block 1)

Compound **36** (22.2 g, 59.0 mmol) and p-nitrophenol (8.23 g, 59.2 mmol) were dissolved in dry dioxane (272 ml) and dry pyridine (14.7 ml). After addition of a solution of N,N'-dicyclohexylcarbodiimide (13.9 g, 67.4 mmol) in dry dioxane (66 ml) the mixture was stirred at room temperature until thin layer chromatography (dichloromethane/ methanol: 9/1, v/v) revealed quantitative conversion (4-18 h). N,N'-Dicyclohexylurea was removed by filtration, the precipitate washed with dioxane until no UV absorbing material could be detected. The solvent was evaporated, the residue azeotropically dried with toluene, dissolved in dichloromethane (70 ml) and remaining dicyclohexylurea removed by filtration. After evaporating the solvent, the residue was directly converted to compound **38**. Remaining DCC could be removed with a small amount of hexane. Yield: 29.1 g (99%). - *^{1}H NMR* (250 MHz, $CDCl_3$):δ = 2.90 (t, 2H, -$C\underline{H}_2$-CH_2-), 3.14 (t, 2H, -CH_2-$\underline{C}H_2$-), 3.78 (s, 6H, -$OC\underline{H}_3$), 5.43 (s, 1H, R_3C-$\underline{H}$), 7.18-6.76 (m, 14H, aryl-$\underline{H}$), 8.22 (d, 2H, O_2N-aryl-$\underline{H}$, ortho). - *^{13}C NMR* (63 MHz, $CDCl_3$): δ = 30.42 (t. -$\underline{C}H_2$-CH_2-), 35.9 (t, -CH_2-$\underline{C}H_2$-), 54.88 (d, $R_3\underline{C}$-H), 55.24 (q, aryl-$O\underline{C}H_3$), 113.69, 122.43, 125.17, 128.31, 129.57, 130.24 (d, $\underline{C}$-H, aryl), 136.39, 137.38, 143.14, 145.32, 155.35 (s, aryl, quarternary), 158.02 (s, $R_2\underline{C}$-OCH_3, aryl), 170.5 (s, -$\underline{C}$OOR).- *MS* (EI): m/z (rel. intensity): m/z calculated for $C_{30}H_{27}NO_6$ (M^+): 497; found: 497 (16), 480 (3), 51 (100). - *MS* (FAB, pos. mode): m/z (rel. intensity): m/z calculated for $C_{30}H_{27}NO_6$ (M^+): 497; found: 497 (26), 466 (4, M-OCH_3^+), 390 (45, M - C_6H_4-OCH_3^+), 375 (24, M - O_2N-$C_6H_4^+$), 227 (100, M - O_2N-C_6H_4-OOC-CH_2-CH_2-$C_6H_4^+$).

Example 6: p-Nitrophenyl-3-{4-[bis-(4-methoxyphenyl)-hydroxymethyl]-phenyl}-propionate (38) (building block 1)

Compound **37** (29.1 g, 58.5 mmol) was dissolved in 670 ml glacial acetic acid and freshly prepared lead dioxide (Rotermund, G.W., *Methoden der organischen Chemie (Houben-Weyl)*, vol. IV/1b, Oxidation, part 2; Georg Thieme Verlag, Stuttgart, 1975, pp. 176) (9.95 g, 41.6 mmol) was added and the mixture placed in a preheated oil

weighed sample was treated either with 5% dichloroacetic acid in dichloromethane (v/v) or with hydrazine reagent IV (table 1) followed by acidifying the solution with 40% trichloroacetic acid in dichloromethane (percentage by weight). The liquid phase was measured at 513 nm (extinction coefficient of an acid solution of the removed trityl derivate: ε = 78600). Amount of nucleoside bound to the support **1**:45.6 µmol/g.

Example 10: Syntheses of the fully deprotected oligonucleotides d(TTTT) and d(TAGCT)

The apparatus for manual oligonucleotide synthesis consisted in a column type reactor fitted with a sintered glass fritt, a stopcock and a connection to a vacuum pump to remove solvents by suction or to dry the support just before the condensation step (step 3, table 2). Only this step was carried out under inert gas athmosphere (argon). The inert gas was introduced to the apparatus via an injection needle through a septum at the top of the apparatus. Another needle through the septum guaranteed equalizing of the gas pressure.

Notes and descriptions of the reagents and solvents:

1) For synthesis 0.0220 g of the support **1** with about 1 µmol loaded nucleoside was used.

2) For the synthesis of d(TTTT) 0.146 g amidite **2a** was dissolved in 1.4 ml acetonitrile (DNA grade). For the synthesis of d(TAGCT) 0.0800 g of **2a-d** each was dissolved in 0.8 ml each of acetonitrile (DNA grade).

3) *Tetrazole reagent:* 31.8 g 1-H-tetrazole in 1 l acetonitrile; GEN 905035.

4) *Oxidation reagent*: 4.3 g iodine in 1 l water/ pyridine/ tetrahydrofuran (THF), 9.05/ 0.41/ 90.54, v/v; GEN 905028.

Table 2. Steps involved in one elongation cycle during synthesis.

Step	Operation	Reagent	Volume (ml)	Duration (min)
1	Delaevulination	Hydrazine reagent	0.7	60
2	Washing	N,N-Dimethylformamide	2 x 5	
		Acetonitrile	2 x 5[a)]	
		Acetonitrile (DNA grade)	1 x 5	
3	Drying	High vacuum (then flushing with argon)		10
4	Condensation	a) Amidite solution	0.4	1[b)]
			0.8	3[c)]
		b) Tetrazole solution	0.8[d)]	9[b)]
			1.6[d)]	10[c)]
5	Washing	Acetonitrile	2 x 5	
6	Oxidation	Oxidation reagent	1.65	1
7	Washing	Acetonitrile	2 x 5	
8	Capping	Capping reagent 1	1.25	1
		Capping reagent 2	1.25	
9	Washing	Acentonitrile	2 x 5	
10	Drying	High Vacuum		some min

a) before washing with acetonitrile (DNA grade) insert septum, b) in case of d(TTTT) synthesis, c) in case of d(TAGCT) synthesis d) add dropwise

Sufficient contact between support and solvent or reagent was guaranteed by occasional gentle shaking, especially after addition of amidite solution and during the dropwise addition of the tetrazole reagent.

1/3 of the solution of step 1 (0.233 ml) was given in a 25 ml standard flask and filled up to the mark with the TCA reagent. The absorptions of the solutions of the elongation cycles were measured spectrophotometrically at 513 nm leading to the nucleoside loading of support **1** and to the yields of the condensation reactions.

Deprotection and purifications of the oligomers:

A) d(TTTT) synthesis:

The support with the attached oligomer was washed with pyridine and the β-cyanoethyl groups were removed with *tert*-butyl amine reagent II (table 1). After washing the support with pyridine and acetonitrile and drying *in vacuo*, the oligomer was removed from the support by treating it with 80% acetic acid for 15 min. After lyophilisation of the solution, the oligomer was purified by HPLC: the terminal 3'-OH protecting group (corresponding to the group of compound **29a** in scheme 7) served here as purification handle. Treatment with 32% ammonia followed by lyophilisation led to the fully deprotected oligomer d(TTTT). - *HPLC:* Ret. time (min): 8.57, UV detection: λ max = 266.1 and 217.7 nm. - *MS* (MALDI-TOF): theoretical mass: $M+H^+$: 1155; found: 1154.

B) d(TAGCT) synthesis:

The oligomer was removed from the support by treatment with 80% acetic acid for 15 min. After lyophilisation, the base, the phosphate and the 3'-OH protection were removed by treatment with 0.5M DBU in acetonitrile leading directly to the fully deprotected d(TAGCT). The reagent was as evaporated *in vacuo*. - *HPLC:* Ret. time (min): 6.96, *UV* detection: λ max= 259.0 and 216.5 nm. - *MS* (MALDI-TOF): $M+H^+$: theoretical mass: 1478; found: 1477.

Deprotection experiments with model compounds

The deprotection experiments with model compounds in solution were monitored by thin layer chromatography. The molar ratios of the deprotection reagents I-IV (table 1) to the model compounds were at least 100: 1.

Claims

1. A process for generating a combinatorial set of molecules of core structure M, comprising the steps of:

(a) preparing a plurality of immobilized molecules of core structure M, wherein said molecules contain a plurality of reactive moieties, each reactive moiety being blocked by a blocking group, wherein at least three of the blocking groups are independently removable under at least three different conditions; and

(b) removing certain blocking groups and derivatizing the resulting reactive moieties in a preprogrammed, regioselective manner, wherein each member of a combinatorial set is uniquely derivatized at at least one reactive moiety with a unique substituent, thereby generating a combinatorial set of molecules of core structure M.

2. A process for generating a combinatorial set of oligomers, comprising the steps of:

(a) preparing a plurality of immobilized molecules of core structure M and containing a plurality of reactive moieties, each reactive moiety being blocked by a blocking group, wherein at least three of the blocking groups are independently removable under at least three different conditions; and

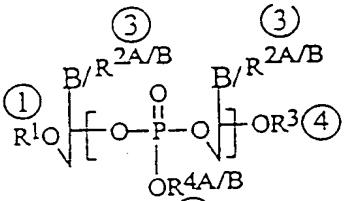
(b) removing certain blocking groups and derivatizing the resulting reactive moieties in a preprogrammed, regioselective manner, wherein at least one reactive moiety is derivatized by addition of a preselected monomer, and said monomer contains a plurality of reactive moieties, each reactive moiety being blocked by a blocking group, wherein at least one of the blocking groups can be independently removed under at least one of the three different conditions;

14. A process of claim 13, wherein the beads are comprised of a material selected from the group consisting of: polystyrene, polyamide, cellulose, Sephadex, Sepharose, silica gel, controlled pore glass (CPG), and teflon.

15. A process of claim 12, wherein the linkage can be cleaved under acidic, alkaline, neutral or photolytic conditions.

16. A process of claim 15, wherein the linkage is selected from the group consisting of tritylether, ester, ß-benzoylpropionyl, levulinyl, disulfide, sulfenyl and derivatives thereof.

17. A composition comprising an oligonucleotide, which is further comprised of monomers of the general formula **49a**:



49a

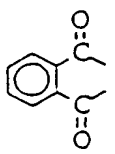
$B/R^{2A/B} = B$ or BR^{2A} or BR^{2B}

$R^{4A/B} = R^{4A}$ or R^{4B}

$R^{2A/B} = R^{2A}$ or R^{2B}

in which ①, ②, ③, ④ are positions in the molecule which can be addressed regioselectively and in which B represents a natural or modified nucleobase, which does not require a protecting group during synthesis, BR^{2A}, BR^{2B} is a natural or modified nucleobase with a protecting group and R^{2A}, R^{4A}, R^1, and R^3 represent different protecting groups that permit the creation of a combinatorial set of oligonucleotides with 16 possible states of protection or subsequent derivatization at positions ① - ④ and R^{2B}, R^{4B} represent protecting groups which are stable during deprotection and/ or subsequent derivatization at ① - ④.

phenoxy}-levulinyl or the linkage to a solid support and $\mathbf{R^1}$ is the 5'-OH functionality selected from the same group as for $\mathbf{R^3}$.



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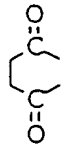

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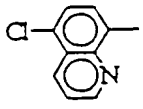
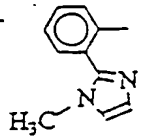


52



53

21. A composition of claim 17, wherein $\mathbf{R^{2A}}$ is 2-nitrophenylsulfenyl (nps), $\mathbf{R^{2B}}$ is 2-(4-nitrophenyl)-ethoxycarbonyl (npeoc) and 2-(4-nitrophenyl)-ethyl (npe) or npeoc, $\mathbf{R^3}$ is 5-{3-[bis-(4-methoxyphenyl)-hydroxymethyl]-phenoxy}-levulinyl or 5-{3-[bis-(4-methoxyphenyl)-methoxymethyl]-phenoxy}-levulinyl, $\mathbf{R^{4A}}$ is ß-cyanoethyl, $\mathbf{R^{4B}}$ is o-/p-chlorophenyl and $\mathbf{R^1}$ is a linkage to a solid support.

22. A composition of claim 21, in which $\mathbf{R^1}$ is a tritylether linkage to Controlled-Pore-Glass (CPG).

23. A combinatorial set of compounds with core structure M and having a plurality of reactive moieties, containing blocking groups, wherein at least three groups are independently removable under different conditions, thereby allowing selective derivatization after deblocking and wherein one functional group is utilized for immobilization.

24. A combinatorial set of compounds according to claim 23 in which the reactive moieties are selected from the group consisting of OH, SH, NH_2, CO_2H, SOH, SO_2H SO_3H, CHO, keto, phosphate, phosphite, phosphoamidite, halogen, CN, CNS, NCS, NCO and derivatives thereof.

25. A combinatorial set of compounds according to claim 23 in which M is a multifunctional low molecular weight compound of general formula MD_n, wherein D represents the same or different independently deprotectable moieties and n an integer from 3 to 10.

26. A combinatorial set of compounds according to claim 23 selected from the group consisting of a: saccharide, aminosugar, deoxysugar, nucleoside, nucleotide, coenzyme, amino acid, lipid, steroid, vitamin, hormone, alkaloid and small molecule drug compound.

27. A combinatorial set of oligomeric compounds according to claim 23 selected from the group consisting of: an oligosaccharide, oligopeptide and oligonucleotide.

28. A combinatorial set of oligomeric compounds of claim 23 in which at one or more positions in the sequence a preselected set of building blocks is incorporated.

29. A combinatorial set of oligomeric compounds according to claim 28 in which different functional groups within each building block in the sequence can be addressed in a sequence specifically preprogrammed way and transformed with protecting or modification reagents in a regioselective form.

30. A combinatorial set of oligonucleotides according to claim 27 which are derived from 2'-deoxyribonucleosides or ribonucleosides.

31. A combinatorial set of oligonucleotides according to claim 30 consisting of natural and/or modified bases selected from the groups adenine, guanine, cytosine, uracil, 5-fluoro-uracil, 5-chloro-uracil, 5-bromo-uracil, 5-iodo-uracil, 5-fluoromethyl-uracil, 5-chloromethyl-uracil, 5-bromomethyl-uracil, 5-iodomethyl-uracil, 5-aminomethyl-uracil, 5-hydroxymethyl-uracil, 5-mercaptomethyl-uracil, 5-fluoro-cytosine, 5-chloro-cytosine, 5-bromo-cytosine, 5-iodo-cytosine, 5-azido-cytosine, 5-alkyl-cytosine, 5-ω-aminoalkyl-cytosine, 5-azido-uracil, 5-alkyl-uracil, 5-ω-aminoalkyl-uracil, 5-methyl-cytosine, 5-amino-cytosine, 5-amino-uracil, 4-triazolo-uracil, 4-triazolo-thymine, 4-tetrazolo-uracil, 4-tetrazolo-thymine, hypoxanthine, xanthine, 2,6-diamino-purine, 6-chloro-purine, 2,6-dichloropurine, 6-thio-purine, 2-thio-purine, 8-fluoro-adenine, 8-chloro-adenine, 8-bromo-adenine, 8-iodo-adenine, 8-fluoro-guanine, 8-chloro-guanine, 8-bromo-guanine, 8-iodo-guanine, 8-amino-adenine, 8-amino-guanine, 8-hydroxy-adenine, 8-hydroxy-guanine, 8-thio-adenine, 8-thio-guanine, N7-deaza-adenine, N7-deaza-guanine, N3-deaza-adenine, N3-deaza-guanine, N9-deaza-adenine, N9-deaza-guanine.

(c) sequentially performing step (b) for the appropriate number of cycles to obtain an oligomer comprising the desired number of monomers, wherein each member of a combinatorial set is uniquely derivatized at at least one reactive moiety with a unique substituent, thereby generating a combinatorial set of oligomers, which are comprised of the same number of monomers, but which differ in the composition of at least one monomer or in the derivatization of at least one reactive moiety within at least one monomer.

3. A process of claim 1, wherein the immobilized molecule is a multifunctional, low molecular weight compound of the general formula MD_n, wherein D represents the same or different independently deprotectable moieties and n is an integer from 3 to 10.

4. A process of claim 3, wherein the low molecular weight compound is selected from the group consisting of a: saccharide, aminosugar, deoxysugar, nucleoside, nucleotide, coenzyme, amino acid, lipid, steroid, vitamin, hormone, alkaloid and small molecule drug compound.

5. A process of claim 2, wherein step (b) is performed for the appropriate number of cycles to obtain an oligomer comprised of a number of monomers in the range of about 2 to about 100.

6. A process of claim 2, wherein the oligomeric compound is selected from the group consisting of: an oligosaccharide, oligopeptide and oligonucleotide.

7. A process of claim 6, wherein the oligonucleotide is derived from a 2'-deoxyribonucleoside and/or a ribonucleoside.

8. A process of claim 7, wherein the oligonucleotide is synthesized in the 3' to 5' or 5' to 3' direction.

9. A process of claim 8, wherein the oligonucleotide is synthesized according to the phosphoamidite, H-phosphonate, or phosphotriester method.

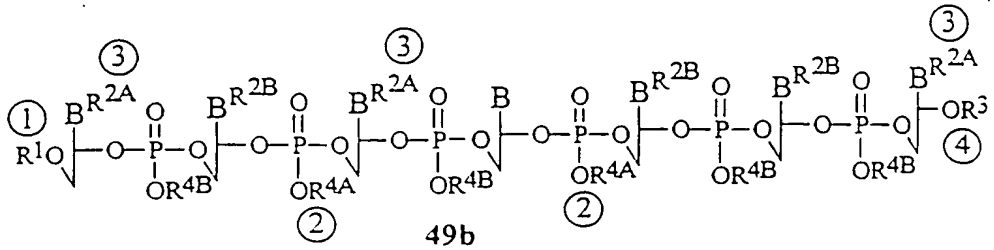
10. A process of claim 6, wherein the oligonucleotide contains a natural or modified base selected from the groups consisting of: adenine, guanine, cytosine, uracil, 5-fluoro-uracil, 5-chloro-uracil, 5-bromo-uracil, 5-iodo-uracil, 5-fluoromethyl-uracil, 5-chloromethyl-uracil, 5-bromomethyl-uracil, 5-iodomethyl-uracil, 5-aminomethyl-uracil, 5-hydroxymethyl-uracil, 5-mercaptomethyl-uracil, 5-fluoro-cytosine, 5-chloro-cytosine, 5-bromo-cytosine, 5-iodo-cytosine, 5-azido-cytosine, 5-alkyl-cytosine, 5-ω-aminoalkyl-cytosine, 5-azido-uracil, 5-alkyl-uracil, 5-ω-aminoalkyl-uracil, 5-methyl-cytosine, 5-amino-cytosine, 5-amino-uracil, 4-triazolo-uracil, 4-triazolo-thymine, 4-tetrazolo-uracil, 4-tetrazolo-thymine, hypoxanthine, xanthine, 2,6-diamino-purine, 6-chloro-purine, 2,6-dichloropurine, 6-thio-purine, 2-thio-purine, 8-fluoro-adenine, 8-chloro-adenine, 8-bromo-adenine, 8-iodo-adenine, 8-fluoro-guanine, 8-chloro-guanine, 8-bromo-guanine, 8-iodo-guanine, 8-amino-adenine, 8-amino-guanine, 8-hydroxy-adenine, 8-hydroxy-guanine, 8-thio-adenine, 8-thio-guanine, N7-deaza-adenine, N7-deaza-guanine, N3-deaza-adenine, N3-deaza-guanine, N9-deaza-adenine, N9-deaza-guanine.

11. A process of claim 1 or 2, wherein the reactive moieties are selected from the group consisting of: OH, SH, NH_2, CO_2H, SOH, SO_2H, SO_3H, CHO, keto, phosphate, phosphite, phosphoamidite, halogen, CN, CNS, NCS, NCO and derivatives thereof.

12. A process of claim 1 or 2, wherein the molecule has been immobilized based on linkage to a solid support.

13. A process of claim 12, wherein the solid support is selected from the group consisting of: beads, flat supports, wafers with or without pits and/or channels, the bottom of a microtiter plate or the inner walls of a capillary.

18. A composition of claim 17, which is of the formula:

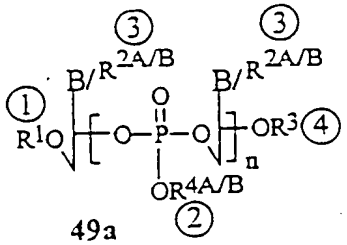


49b

19. A composition of claim 17, wherein the oligonucleotide is deprotected or derivatized in 64 different regioselective combinations, by using 2-nitrophenylsulfenyl as R^{2A} for the protection of adenine (A), cytosine (C) or guanine (G).

20. A composition of claim 17 or 18 in which $\mathbf{R^{2A}}$ or $\mathbf{R^{2B}}$ is a base protection group selected from the group consisting of an acyl group, which is selected from the group consisting of: acetyl, benzoyl, anisoyl, p-/o-tolyl, phenoxyacetyl, t-butylphenoxyacetyl or 2-nitrophenylsulfenyl (nps), 2-(4-nitrophenyl)-ethoxycarbonyl (npeoc), 2-(4-nitrophenyl)-ethyl (npe), 9-fluorenylmethoxycarbonyl (Fmoc), benzyloxycarbonyl, benzyl, allyloxycarbonyl, N,N-dimethylaminomethylidene (DMM) and derivatives thereof, p-nitrobenzylidene, levulinyl, compound **50** or a derivative thereof, compound **51**, trityl, monomethoxytrityl, dimethoxytrityl, trimethoxytrityl, 2,2,2-trichloro-*tert*-butyloxycarbonyl (TCBOC), $\mathbf{R^{4A}}$ or $\mathbf{R^{4B}}$ is a phosphate protecting group selected from the group consisting of an alkyl, aralkyl, allyl, ß-cyanoethyl, o-/-p-chlorophenyl, 2,5-dichlorophenyl, trichloroethyl, tribromoethyl, sulfonylethyl or derivatives thereof, 4-*tert*-butyl-2-chloro-phenyl, phenylmethylamino, 2,4-dichlorophenyl, m-chlorophenyl, o-fluorophenyl, benzyl, benzhydryl, ß,ß,ß-trichloroethyl, 4-nitro-2-chloromethyl-phenyl, 2-(4-nitrophenyl)-ethyl (npe), 4-nitrophenyl, compound **52** and **53**, $\mathbf{R^3}$ is the 3'-OH functionality, selected from the group consisting of trityl, substituted trityl, triphenylmethoxyacetyl, diphenyl-*tert*-butylsilyl, succinyl, ß-benzoylpropionyl, levulinyl, *tert*-butyl-dimethyl-silyl, 2,4-dinitrophenylsulfenyl (dnps), 9-fluorenylmethoxycarbonyl (Fmoc), 3-{4-[bis-(4-methoxyphenyl)-methyl]-phenyl}-propionyl, 5-{3-[bis-(4-methoxyphenyl)-hydroxymethyl]-phenoxy}-levulinyl, 5-{3-[bis-(4-methoxyphenyl)-methoxymethyl]-

32. A combinatorial set of oligonucleotides according to claim 31 represented by the general formula **49a**:



49a

$B/R^{2A/B} = B$ or $B^{R^{2A}}$ or $B^{R^{2B}}$

$R^{4A/B} = R^{4A}$ or R^{4B}

$R^{2A/B} = R^{2A}$ or R^{2B}

in which n is an integer from 1 to 100 and ①, ②, ③, ④ are positions in the molecule which can be addressed regioselectively and in which B represents a natural or modified nucleobase which does not need a protecting group during synthesis, $_BR^{2A}$, $_BR^{2B}$ is a natural or modified nucleobase with a protecting group and R^{2A}, R^{4A}, R^1 R^3 represent different protecting groups that permit the creation of a combinatorial set of oligonucleotides with 16 possible states of protection or subsequent derivatization at ①- ④ and R^{2B}, R^{4B} represent protecting groups which are stable during deprotection and/ or subsequent derivatization at ①- ④.

33. A combinatorial set of oligonucleotides according to claim 32 in which R^{2A} or R^{2B} is a base protection group selected from the group of acyl groups such as acetyl, benzoyl, anisoyl, p-/o-tolyl, phenoxyacetyl, t-butylphenoxyacetyl or 2-nitrophenylsulfenyl (nps), 2-(4-nitrophenyl)-ethoxycarbonyl (npeoc), 2-(4-nitrophenyl)-ethyl (npe), 9-fluorenylmethoxycarbonyl (Fmoc), benzyloxycarbonyl, benzyl, allyloxycarbonyl, N,N-dimethylaminomethylidene (DMM) and derivatives thereof, p-nitrobenzylidene, levulinyl, compound **50** or derivatives thereof, compound **51**, trityl, monomethoxytrityl, dimethoxytrityl, trimethoxytrityl, 2,2,2-trichloro-*tert*-butyloxycarbonyl (TCBOC), R^{4A} or R^{4B} are phosphate protecting groups such as alkyl, aralkyl, allyl, ß-cyanoethyl, o-/-p-chlorophenyl, 2,5-dichlorophenyl, trichloroethyl, tribromoethyl, sulfonylethyl or derivatives thereof, 4-*tert*-butyl-2-chloro-phenyl, phenylmethylamino, 2,4-dichlorophenyl, m-chlorophenyl, o-fluorophenyl, benzyl, benzhydryl, ß,ß,ß-trichloroethyl, 4-nitro-2-chloromethyl-phenyl, 2-(4-nitrophenyl)-ethyl (npe), 4-nitrophenyl, compound **52** and **53**, R^3 is the 3'-OH functionality, which can be a

trityl, substituted trityl, triphenylmethoxyacetyl, diphenyl-*tert*-butylsilyl, succinyl, ß-benzoylpropionyl, levulinyl, *tert*-butyl-dimethyl-silyl, 2,4-dinitrophenylsulfenyl (dnps), 9-fluorenylmethoxycarbonyl (Fmoc), 3-{4-[bis-(4-methoxyphenyl)-methyl]-phenyl}-propionyl, 5-{3-[bis-(4-methoxyphenyl)-hydroxymethyl]-phenoxy}-levulinyl, 5-{3-[bis-(4-methoxyphenyl)-methoxymethyl]-phenoxy}-levulinyl or the linkage to a solid support and $\mathbf{R^1}$ are 5'-OH functionalities selected from the same group as $\mathbf{R^3}$.

34. A combinatorial set of oligonucleotides according to claim 32 in which $\mathbf{R^{2A}}$ are the 2-nitrophenylsulfenyl (nps) groups, for $\mathbf{R^{2B}}$ the 2-(4-nitrophenyl)-ethoxycarbonyl (npeoc) and 2-(4-nitrophenyl)-ethyl (npe) or npeoc groups, for $\mathbf{R^3}$ the 5-{3-[bis-(4-methoxyphenyl)-hydroxymethyl]-phenoxy}-levulinyl or 5-{3-[bis-(4-methoxyphenyl)-methoxymethyl]-phenoxy}-levulinyl groups, for $\mathbf{R^{4A}}$ the ß-cyanoethyl groups, for $\mathbf{R^{4B}}$ the o-/p-chlorophenyl groups and $\mathbf{R^1}$ is a linkage to a solid support.

35. A combinatorial set of oligonucleotides according to claim 32, in which $\mathbf{R^1}$ is a tritylether linkage to Controlled-Pore-Glass (CPG).

36. A combinatorial set of oligonucleotides according to claim 32, which have been synthesized using the phosphoamidite, H-phosphonate or phosphotriester synthesis method.